

Gypsy moths (Lymantria dispar) in the Niagara Region:
population density variation, introduction of an
entomopathogenic fungus (Entomophaga maimaiga)
and occurrence of nuclear polyhedrosis virus

by

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Abstract

The gypsy moth, Lymantria dispar, a major defoliator of broadleaf trees, was accidentally introduced into North America in 1869. Much interest has been generated regarding the potential of using natural pathogens for biological control of this insect. One of these pathogens, a highly specific fungus, Entomophaga maimaiga, was accredited with causing major epizootics in populations of gypsy moth across the north-eastern United States in 1989 and 1990 and is thought to be spreading northwards into Canada. This study examined gypsy moth population densities in the Niagara Region. The fungus, E. maimaiga, was artificially introduced into one site and the resulting mortality in host populations was noted over two years. The relationship between fungal mortality, host population density and occurrence of another pathogen, the nuclear polyhedrosis virus (NPV), was assessed.

Gypsy moth population density was assessed by counting egg masses in 0.01 hectare (ha) study plots in six areas, namely Louth, Queenston, Niagara-on-the-Lake, Shorthills Provincial Park, Chippawa Creek and Willoughby Marsh. High variability in density was seen among sites. Willoughby Marsh and Chippawa Creek, the sites with the greatest variability, were selected for more intensive study.

The pathogenicity of E. maimaiga was established in laboratory trials. Fungal-infected gypsy moth larvae were then released into experimental plots of varying host density in Willoughby Marsh in 1992. These larvae served as the inoculum to infect field larvae. Other larvae were injected with culture medium only and released into control plots also of varying host density. Later, field larvae were collected and assessed for the presence of E. maimaiga and NPV. A

greater proportion of larvae were infected from experimental plots than from control plots indicating that the experimental augmentation had been successful. There was no relationship between host density and the proportion of infected larvae in either experimental or control plots. In 1992, 86% of larvae were positive for NPV. Presence and intensity of NPV infection was independent of fungal presence, plot type or interaction of these two factors.

Sampling was carried out in the summer of 1993, the year after the introduction, to evaluate the persistence of the pathogen in the environment. Almost 50% of all larvae were infected with the fungus. There was no difference between control and experimental plots. Data collected from Willoughby Marsh indicated that there was no correlation between the proportion of larvae infected with the fungus and host population density in either experimental or control plots. About 10% of larvae collected from a nearby site, Chippawa Creek, were also positive for E. maimaiga suggesting that low levels of E. maimaiga probably occurred naturally in the area. In 1993, 9.6% of larvae were positive for NPV. Again, presence or absence of NPV infection was independent of fungal presence plot type or interaction of these two factors.

In conclusion, gypsy moth population densities were highly variable between and within sites in the Niagara Region. The introduction of the pathogenic fungus, E. maimaiga, into Willoughby Marsh in 1992 was successful and the fungus was again evident in 1993. There was no evidence for existence of a relationship between fungal mortality and gypsy moth density or occurrence of NPV. The results from this study are discussed with respect to the use of E. maimaiga in gypsy moth management programs.

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Introduction

The gypsy moth, Lymantria dispar, is a major foliage pest of deciduous forests in the northern hemisphere (Elkinton and Liebhold, 1990). Since its accidental introduction into North America in 1869, many millions of dollars have been spent on mainly chemical methods of control (Dreistadt and Weber, 1989). Chemical insecticides, while reasonably effective, are costly, short-lasting and often toxic to non-target organisms. Biological control agents, such as predators, parasitoids and/or natural pathogens can be used as an alternative or a supplement to chemical control agents. Effective biological control agents are host-specific and persist in the environment, thus obviating the need for repeated applications.

Several natural enemies of the gypsy moth have been introduced into North America since the turn of the century with mostly limited success. Much interest presently centres around an entomopathogenic fungus and a virus. The fungus, named Entomophaga maimaiga, is a major mortality agent of gypsy moth in Japan (Aoki, 1974; Shimazu and Soper, 1986; Soper et al., 1988) and has been introduced into North America (Speare and Colley, 1912; Soper et al., 1988). Interest in E. maimaiga was revived in 1989 when it was accredited with causing major epizootics in populations of gypsy moth in New England between 1989 and 1990 (Andreadis and Weseloh, 1990; Elkinton et al., 1991) and since then it has expanded its range and been reported from eastern Ontario (Welton, 1991). E. maimaiga does not seem to spread naturally as quickly as the gypsy moth. Gypsy moth populations are obviously spreading rapidly and it is known that this fungus can be a major mortality factor. Thus, any means by which the introduction of

this naturally occurring pathogen can be accelerated should be investigated.

Another mortality agent, the gypsy moth nuclear polyhedrosis virus (NPV), has been introduced into North America and is considered an important entomopathogen (Elkinton and Liebhold, 1990). Both pathogens can often occur in the same host population. In the past fungal epizootics may have, in fact, been mistaken for virally-caused epizootics because gypsy moth larvae killed by either of these pathogens look similar. Both pathogens may in fact occur within the same individual (Hajek and Roberts, 1992) but little is known about possible interactions between them.

The purpose of this research was: to collect data regarding gypsy moth population densities in different locations in the Niagara Peninsula; to determine if E. maimaiga could be introduced into Willoughby Marsh Conservation Area by artificial inoculation and release of infected larvae; to study the relationship between moth density and fungal-caused mortality; to determine the persistence of the released isolate of E. maimaiga in the release area; and also to examine the relationship between occurrence of E. maimaiga and occurrence and intensity of NPV infection.

Literature Review

There are two main sections in the following literature review. In the first section, important aspects of the biology of the European gypsy moth and various physical, chemical and biological control methods are reviewed. In the second section, current knowledge regarding a specific pathogen of gypsy moth, Entomophaga maimaiga, is discussed.

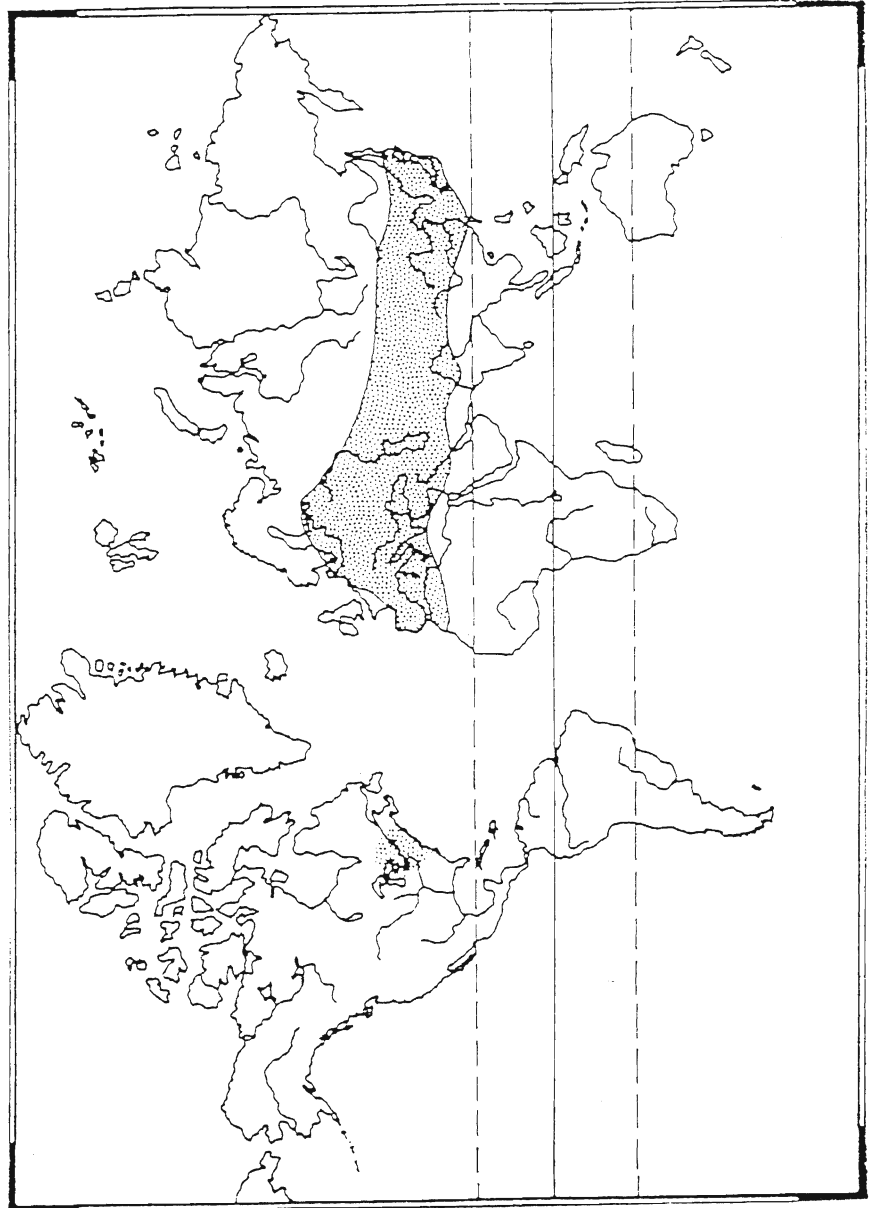
The European Gypsy Moth

The European gypsy moth, Lymantria dispar (Linnaeus) (Lepidoptera: Lymantriidae), is the most important defoliator of hardwood forests in the northeastern United States (Andreadis and Weseloh, 1990). This destructive species has expanded its range into Canada and may now be found as far north as Sault Ste. Marie (Howse and Applejohn, 1992) and as far west as British Columbia (Benoit and Lachance, 1990).

Introduction and Spread in North America

The gypsy moth is endemic to northern portions of Africa and to western and eastern Europe and is now found in North America (Figure 1). It was first introduced into North America from Europe in the late 1860s (Forebush and Fernald, 1896) when some specimens were imported into Medford, Massachusetts by Léopold Trouvelot. Trouvelot was attempting to produce hardier varieties of silkworms by cross-breeding them with other lepidopterans. A few gypsy moths accidentally escaped into nearby woodlots and populations of the pest rapidly grew and flourished. In the years to follow, the social and economic costs engendered by this species was enormous (Leonard, 1981).

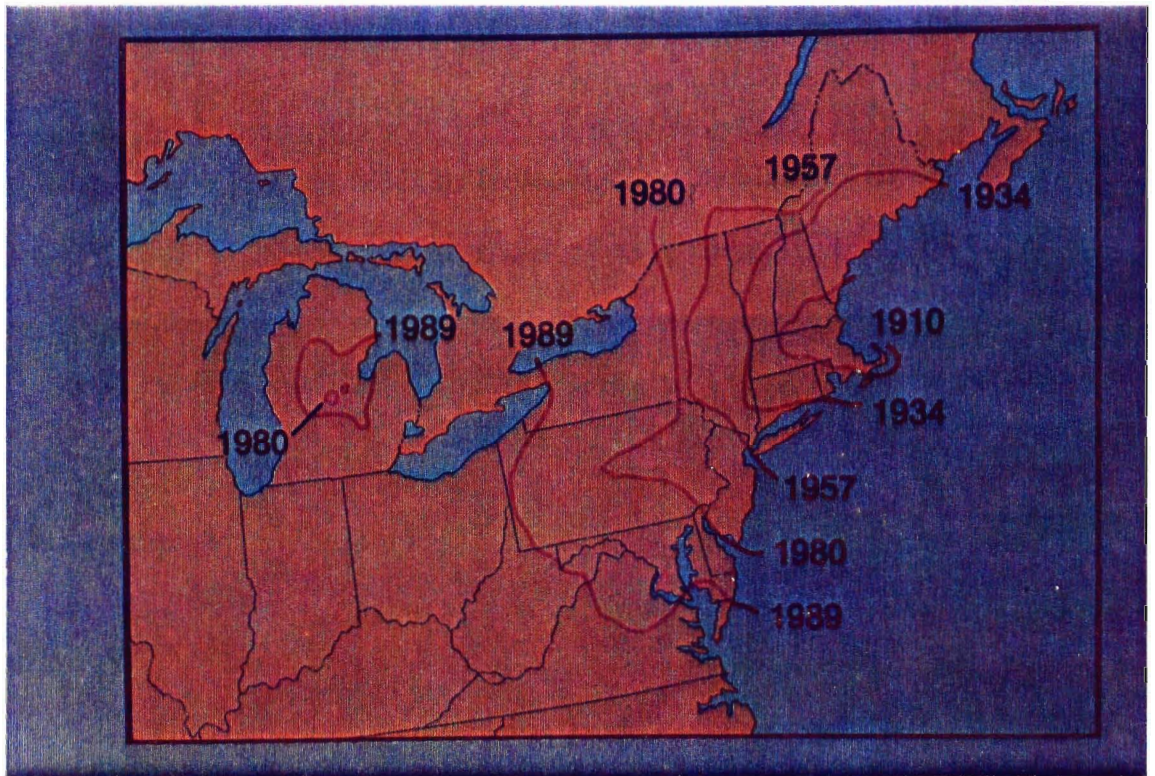
Figure 1. Distribution of the gypsy moth, L. dispar
(Dreistadt and Weber, 1989).



Historical data compiled to document the invasion process of gypsy moth into North America indicate a high rate of population expansion from 1900 until 1915, a slow rate from 1916 until 1965 and a greatly accelerated rate since 1966. Explanations for the different rates may be associated with geographical and temporal changes in mean minimum temperature, the varying distribution of forest species composition and differing levels of human intervention. Increased recreational use of forests and subsequent transport of egg masses on recreational vehicles may account for the increased rate of expansion since 1966 (Elmes, 1992). Spread of gypsy moth by humans has resulted in isolated infestations well beyond the eastern seaboard in California, Washington, Oregon (McManus and McIntyre, 1981) and British Columbia (Benoit and Lachance, 1990).

Invasion of gypsy moth into North America radiated from the point of introduction in Medford, Massachusetts south and west and finally north into Canada (Figure 2). Gypsy moths spread into southern Quebec from New York state by 1959 and, in 1969, appeared for the first time in Ontario near Kingston (Benoit and Lachance, 1990). Populations of the pest expanded and spread but levels of destruction were not uniform in all years due to the cyclic nature of gypsy moth population dynamics. Between 1982 and 1987, only around one percent of the annual tree mortality rate in Ontario was caused by the gypsy moth. This amount of mortality is probably similar to that which would be expected in hardwood stands growing on sites with sub-optimum characteristics. During this period, most damage caused by gypsy moth in Ontario occurred between 1985 and 1987 and the eastern region of the province experienced the heaviest infestations. This damage in the east reflects the heavy impact of an introduced

Figure 2. Dispersal of gypsy moth in northeastern North America since 1869 (Elmes, 1992).

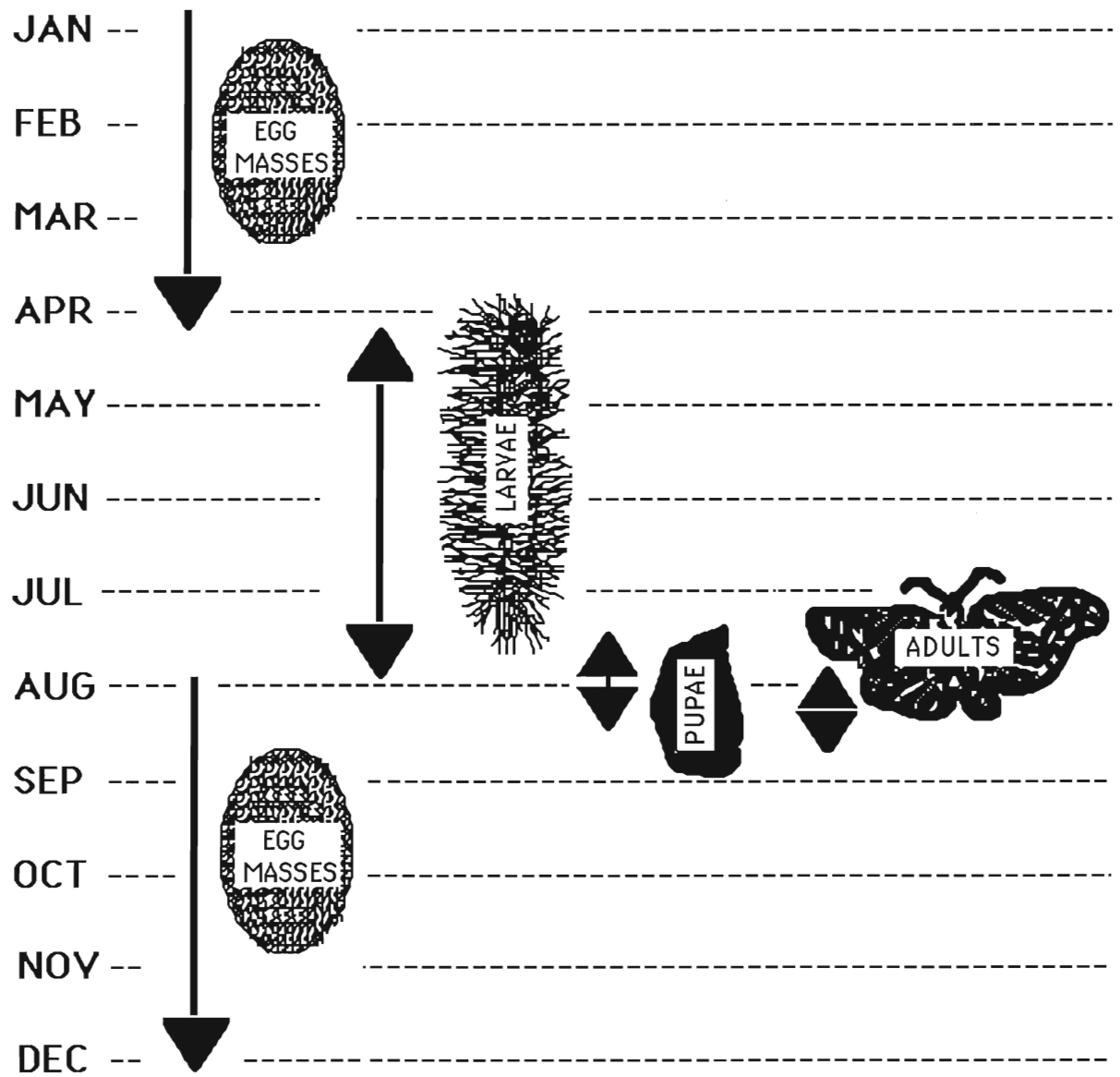


pest early in an infestation when food may be plentiful and natural enemies scarce (Gross et al., 1992). Populations increased in Ontario between 1988 and 1989 but decreased in many parts of the province in 1990 due to high overwintering mortality that occurred in the southeastern part of the province from Toronto to the Quebec border. However, gypsy moth populations did increase in the Niagara Region and areas of defoliation occurred for the first time in Algonquin Park, the Muskokas, and on Manitoulin Island (Churcher, 1990). In 1991, population levels increased again, except in areas affected by the oldest part of the outbreak, that is in the eastern region and in the extreme southwestern part of the province (Howse and Applejohn, 1991). Dramatic declines in defoliation occurred in 1992 in most parts of Ontario except where populations were in the process of invading new territory i.e. around Sudbury and Sault Ste. Marie (Howse and Applejohn, 1992).

Life Cycle

The gypsy moth is univoltine and the four stages of its life cycle are egg, larva, pupa and adult (Figure 3). Gypsy moth egg masses are laid in mid- to late summer. Eggs are all laid in a single mass containing from 100 to 1000 eggs. Masses contain more eggs at low than at high population densities. Egg masses are covered with buff-coloured abdominal hairs from the females body giving them the appearance of a sponge. Pale yellow to pink when first laid, eggs turn brown or dark brown as embryos develop. Partial development occurs in the first three weeks following oviposition, ceases over the winter and then continues the following spring. Larvae chew through the chorion of the egg shell and hatch out in mid-spring. The hatching

Figure 3. Life cycle of the gypsy moth, L. dispar, in Ontario.



phase can last from ten to 30 days. Hatching is strongly influenced by temperature (Leonard, 1981).

The duration of each successive instar depends on environmental conditions. The first instar generally lasts between five and ten days. The next three or four instars last about a week each and the final instar lasts between ten and 15 days. Male and female larvae usually undergo five and six instars respectively although the numbers may vary considerably. Several factors, including the quantity and quality of food, population density and low temperatures often cause additional instars, especially during the early stages of development (Benoit and Lachance, 1990).

The pupal stage of gypsy moth may continue for as long as 17 days but on average it lasts ten days for a female and 13 for a male (Benoit and Lachance, 1990). Males pupate about a week before females but as they spend a longer time in this stage, they eclose only one to three days before females (Montgomery and Wallner, 1988). Male moths (35-45mm), with their plumose antennae, are dark brown and their forewings are highlighted by several dark brown lines, marks and splotches. The larger females (40-50mm) are predominantly white to yellowish-white and they have brown to black markings on the forewings. Females rarely fly. Newly eclosed females emit a pheromone called disparlure which attracts the male. Males fly in a zigzag pattern following the trail of the pheromone plume. A male can successfully inseminate several females, but a female usually mates only once (Benoit and Lachance, 1990). The oviposition site is usually within one or two metres of the female pupation site (Montgomery and Wallner, 1988).

Feeding Ecology

The gypsy moth feeds only during the larval stage and is considered a generalist herbivore, feeding on a wide variety of plant species (Montgomery and Wallner, 1988). Gypsy moth larvae prefer oak (Quercus spp.), but populations have become established on other tree species including trembling and bigtooth aspen (Populus tremuloides Michaux and P. grandidentata Michaux). Foliage of red maple (Acer rubrum L.) is not favoured and that of the tulip tree (Liriodendron tulipifera L.) is rejected. However, during a major population outbreak, all tree species are vulnerable to attack by gypsy moth. It is estimated that one larva consumes about one m² of foliage during its development. Trees under stress such as drought contain higher levels of nitrogen which may provide larvae with an enriched food source. Stressed trees may be chosen by moths. The foci of epidemic populations are often found on dry, rocky ridges where conditions for tree growth are not optimum. Trees under prolonged stress may not recover to refoliate the next year and thus recurring gypsy moth infestations may cause high tree mortality (Leonard, 1981).

Larval behaviour changes with developmental stage and population density. Early instar larvae are very specific in their host preferences, while later instars feed on a wide range of host plants (Elkinton and Liebhold, 1990). Older instars feed mostly at night and move off the treetops during the daytime to shelter in crevices or cracks in the bark, or in ground cover (Gerardi and Grimm, 1979). This pattern, however, changes with variations in population density. At high population densities, older larvae feed both day and night (Lance et al., 1986).

Dispersal

The gypsy moth has four primary methods of dispersal. Chief among them is the act of ballooning by first instars (Mason and McManus, 1981). First instar larvae use silk lines to descend away from the trees they hatched on. Larvae also possess dorsal aerostatic and lateral acuminate hairs which increase their surface area and make them buoyant. The numerous hairs, both long and tapered, and short and bulbous, give neonates a very high soaring coefficient. This coefficient is a measure of a body's ability to be carried by an air current. The soaring coefficient of a newly hatched larva is comparable to that of a highly efficient airborne seed, such as that of the dandelion or of the milkweed. Wind-borne caterpillars may be active for a period of 20-30 days. Maximum dispersal occurs between the 10th and 15th day following the start of hatching when warm weather is accompanied by winds of at least four km/hr. Dispersal is optimum when temperatures are between 20°C and 30°C and winds are eight km/h or greater (Benoit and Lachance, 1990). Local outbreak populations usually collapse after a year or two but dispersal of first instars between stands can maintain high density populations on a regional scale for up to a decade (Campbell and Sloan, 1978a).

The three other modes of dispersal occur during the late larval, the adult and the egg stages of the life cycle. Late instar larvae are very mobile and may move as much as 250m from one feeding site to another over the course of a season (Hajek, pers. comm.). Male gypsy moth adults often fly into new areas and search for mates. Egg masses may be inadvertently spread into new areas if they are deposited on items such as outdoor furniture, timber, or recreational vehicles that are destined for transport by humans.

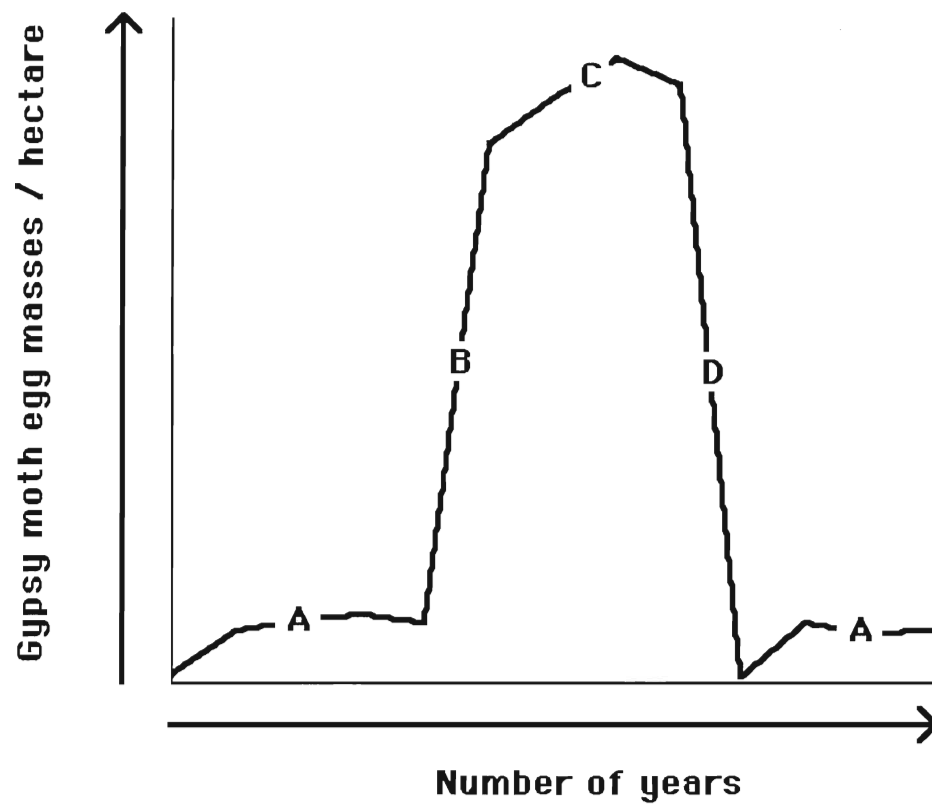
Population dynamics

Population dynamics of gypsy moth in Europe have often been described as cyclic. Increases from low to high densities occur every seven to nine years with periodic outbreaks lasting for about three years (Dobrivojevic, 1963). In contrast to this cyclic pattern, gypsy moth population systems in North America characteristically display a numerically bimodal pattern (Campbell and Sloan, 1978b). This pattern typically has four distinct features, two relatively stable phases called innocuous and outbreak, and two transient phases called release and decline (Figure 4). Innocuous population densities range from two to 25,000 fourth instar larvae per hectare and the innocuous phase can last indefinitely (Campbell, 1981).

A low density or innocuous population can occasionally expand from within relatively small and discrete focal areas during a release phase (Campbell, 1981). Sparse populations tend to be released when they are close to human activities (Campbell *et al.*, 1976). Some outbreaks originate in sites where naturally occurring, protected locations (e.g. bark flaps) or their equivalent (man-made objects) provide abundant resting sites above the forest floor where gypsy moths are protected from harsh environmental conditions and predators. Trees with man-made objects have several times more egg masses than trees with natural hiding places. Trees with man-made objects along the forest edge have higher egg mass densities than similar trees within the forest (Campbell, 1981). High egg mass density and high egg survival may lead to rapid growth of a population. A key weather event that adversely influences growth of gypsy moth populations may be a series of extremely harsh winters. Weather events that promote growth of populations may be an absence of cold

Figure 4. A hypothetical North American gypsy moth population displaying numerical bimodality.

A = Innocuous mode
B = Release phase
C = Outbreak mode
D = Decline phase



weather in mid-spring or a series of hot, dry summers. Population expansion may also be related to meteorological conditions which may reduce predation and disease of gypsy moth (Vasic, 1958). Bess *et al.* (1947) suggested that land abuses (fire, repeated clear-cutting and overgrazing) and drought contribute to favourable conditions for gypsy moth outbreaks.

Populations of gypsy moth are capable of sustained outbreaks that can last from one to many years. An outbreak often follows shortly after initial invasion. Outbreak population densities range from 250,000-2,500,000 fourth instar larvae per hectare. Individual subpopulations in areawide outbreaks can reach up to 12,500,000 fourth instar larvae per hectare. Outbreaks continue to occur after the original invasion but they are usually less spectacular and damaging than the original one (Campbell, 1981). Gypsy moth populations in North America show a tendency for explosive increases near the advancing front of a generally infested area. Fecundity may be exceptionally high near the advancing front of the generally infested area as a result of the high degree of food quality associated with unstressed plants (Campbell, 1981). Because of the high fecundity of gypsy moths, mortality in immature stages in excess of 80% may still not be sufficient to cause gypsy moth population decline. In a field study performed by Hajek *et al.* (1990a), mortality among late-instar larvae ranged from 60% to 88%. Despite these high levels of mortality, there was relatively little change the next year in gypsy moth egg mass densities.

Repeated defoliation by gypsy moth can result in dramatic forest responses in North America which reduce the capacity of the forest to sustain an outbreak. These responses include decreased nutritive

quality of leaves, reduced ability to refoliate and even death of trees. Parasites and predators usually play a minor role in the decline of gypsy moth outbreaks. Areawide population collapse usually results from the widespread occurrence of overpopulation phenomena, notably disease, decreased fecundity and starvation (Campbell, 1981).

Estimating Population Density

Methods have been developed to census different life stages of gypsy moth to obtain estimates of population density. Larval density may be estimated by collecting, measuring and counting head capsules (Higashiura, 1987) or by measuring frass production (Liebhold and Elkinton, 1988). Density of adult gypsy moths may be estimated by trapping male moths in pheromone traps (Gage *et al.*, 1990). However, the density of egg masses per unit area is considered to be the most reliable means for estimating population density and predicting population trends (Leonard, 1981). Egg masses are immobile and are present for several months of the year from late summer to mid-spring. Each female gypsy moth generally lays one egg mass; only rarely does a female deposit eggs in more than one mass. The oviposition site varies depending on the density of the gypsy moth population. At high population densities, most egg masses are deposited on tree trunks and branches. At low population densities, they are deposited closer to the forest floor, on structures such as rocks and logs (Elkinton and Liebhold, 1990).

Density estimates using egg masses are accomplished by counting all those that occur within certain prescribed areas e.g. fixed- and variable-radius plots (Wilson and Fontaine, 1978) or plots of standard area (0.01, 0.05, or 0.61ha) (Kolodny-Hirsch, 1986). Egg mass density is then expressed as numbers per unit ground area (e.g.

number per hectare). Density estimates can be used to predict defoliation levels using one of several previously developed density-defoliation relationships (Liebhold et al., 1993). Egg mass density is the primary decision-making variable in many gypsy moth integrated pest management programs in the United States (Fleischer et al., 1991). Counts have been used extensively by other workers. Bellinger et al. (1989) used egg mass counts to examine the influence of forest edge effects on egg mass distribution. Gould et al. (1990) used egg mass counts in determinations of changes in gypsy moth density induced by the actions of natural enemies. Hajek and Roberts (1991) used egg mass counts to select determine changes in density after the introduction of an entomopathogenic fungus. Egg mass counts are used in surveys conducted by the Ontario Ministry of Natural Resources (Nealis and Erb, 1993) and were chosen as an effective means of estimating population density in this study.

Control of Gypsy Moth Populations

The gypsy moth currently inhabits only one-fifth of its potential range in North America and there is no evidence that suppression activities have reduced its rate of expansion (Elmes, 1992). Control of gypsy moth populations can occur in many ways. Various physical, chemical and biological control methods can be used with varying degrees of success.

Physical and Chemical Methods

Within a few years of its introduction into North America, both the size of the gypsy moth population and the area of the forest affected by it had increased dramatically. Concerned authorities hastened to devise and implement physical methods of control. Egg masses were burned. Infested trees were cut down. However, these

attempts to eradicate the pest proved fruitless (Metterhouse, 1989). Physical methods are still employed today as methods of control rather than of eradication. Sticky tape and burlap bands, applied around a tree trunk, act as a barrier to larvae crawling up and down. Intercepted larvae can then be physically destroyed. As well, roadside checks on vehicles and lumber are performed in order to prevent accidental transport of egg masses or larvae.

More pounds of pesticides have been applied against the gypsy moth than against any other exotic pest with the possible exception of the spruce budworm, Choristoneura fumiferana (Clemens) (NRC, 1975). The first insecticide to be used to kill gypsy moth was Paris green. It was replaced by lead arsenate, a compound developed specifically for this pest. From the middle 1940s until the late 1950s, DDT was used extensively against the gypsy moth. For example, in 1957 1.2 million hectares were sprayed aerially with DDT. However, due to increasing public concern, its use was discontinued in 1958 and it was replaced in the United States by Carbaryl (Sevin®) (McManus and McIntyre, 1981). Carbaryl is federally registered in Canada and provincially approved for ground and aerial application in the control of gypsy moth in forested areas. This insecticide acts both externally and internally as a contact poison and as a stomach poison respectively. It is a synthetic organic carbamate insecticide toxic to many insects directly exposed to spray residues, including bees, aquatic species and beneficial predators and parasitoids of the gypsy moth (OMNR, 1991). Another insecticide in use at present is Dimilin®, the trade name of the insect growth regulator, diflubenzuron. It inhibits chitin biosynthesis; at the initiation of ecdysis the cuticle of treated larvae is only partially formed and is improperly attached to the epidermis.

While it has an effect on some other species of chitin-containing invertebrates, it is rapidly degraded (White et al., 1981).

Synthetically produced pheromones or insect hormones are also utilized in efforts to control gypsy moth. They are used principally in initiation and evaluation of eradication methods. Gyplure, widely used for detection purposes from 1960-1970, was misidentified as a pheromone. Its use was discontinued when applications proved to be ineffective. It was replaced by disparlure in 1972 (Dreistadt and Weber, 1989). Because disparlure is potent and long-lasting, pheromone-baited traps are effective for several months (Elkinton and Cardé, 1980). Low level infestations may be undetectable using the typical trap densities used in extensive trapping. Additional localized trapping may therefore be required in an area designated for a proposed eradication (Dreistadt and Weber, 1989).

Another potential avenue of suppressing gypsy moth populations is through the release of sterile male moths. Since a female gypsy moth generally only mates once, a mating with a sterile male will produce unfertilized eggs. Field trials are being conducted on three fronts, release of fully sterile males released as pupae, release of sub-sterile males introduced as pupae which produce sterile F1 offspring and introduction of F1 sterile moths in egg masses. The latter method is preferred since it is the least costly (Dreistadt and Weber, 1989).

Biological Control Agents

Increasing public awareness of the potential health hazards associated with chemical insecticides has promoted interest in biological control. There are several advantages and disadvantages associated with biological control. Good natural enemies used in

biological control have little or no effect on non-target organisms. Host-specific natural enemies respond more precisely to changes in host density than more polyphagous ones. However, when pest populations are low, more general predators may have the advantage. Effective natural enemies are able to seek out and find the target organism once released into host populations. If the pest population is to be kept at low levels, the natural enemy must continue to search rather than to emigrate from the area when the host becomes scarce. Effective natural enemies will propagate and spread. They should be able to survive in all niches and throughout the climatic range occupied by the pest. They should be self-perpetuating once released. High potential increase rate, a short development time, large numbers of generations per year and high fecundity are good attributes of a natural enemy used against a pest with similar properties. Natural enemies require no manufacturing process but they should be easy to culture for inundation and inoculation procedures. Slow development of resistance in the target organism usually occurs when natural enemies are used. Biological control agents leave no toxic residues in the environment and they may be compatible with toxic chemicals and sometimes combined with them in a spray. They have a low dosage requirement and are therefore cheap and versatile. However, biological control is usually a slow process and pest populations are not eradicated but are rather maintained at low levels (van Emden, 1974).

Although efforts of eradication and suppression of gypsy moth continue, they have thus far been relatively unsuccessful. Reliance on insecticidal control of the gypsy moth has been replaced in recent years by a broader integrated pest management approach. One facet of

this approach is to study naturally-occurring predators and pathogens such as various species of birds, small mammals, insects, and micro-organisms and to assess their potential for a role in integrated pest management. Use of these enemies against gypsy moth will be explored in the following sections of this literature review.

Vertebrates and Invertebrates

Vertebrate predators of gypsy moth include birds and small mammals. Low density population levels of gypsy moth may be maintained by the combined predatory efforts of some species of birds and small mammals. Predaceous birds are a primary cause of mortality among fifth and sixth larval instars. Small mammals are a primary cause of mortality among pupae. Vertebrate predation may be an essential component in the control of innocuous populations of gypsy moth (Campbell and Sloan, 1977). Invertebrate control agents of gypsy moth include ants, ground beetles, and many dipteran and hymenopteran parasitoids. It is difficult to measure the effect of invertebrate predators and parasitoids on gypsy moth populations because, in comparison to vertebrate predators, little is known about them (Smith and Lautenschlager, 1988). What is known about a few of them will be briefly discussed later in this section.

Furuta (1976) cites birds as the most important factor in maintaining gypsy moth populations at low levels in Japan where bird predation of egg masses may be as important a mortality factor as parasitism in egg and larval stages of L. dispar. The most common avian predator of egg masses is the nuthatch, Sitta spp. The behaviour of birds attacking egg masses suggests that the egg masses are unpalatable. In a field study it was observed that birds consume the clusters, not wholly, but bit by bit. Egg masses are eaten mainly

during winters with heavy snowfall. Egg masses deposited on the upper part of trunks are consumed before the heavy snow period. During the intensive predation period, February to early March, birds extend their foraging sites downward and attack egg masses on the lower parts of tree trunks. Since those masses that were to be found below the snow line escape predation, the height of the oviposition site selected by the female gypsy moth can affect the survival of egg masses (Higashiura, 1989a). In regions of low snowfall almost all egg masses are deposited in tree canopies, frequently on undersides of branches. In regions of higher snowfall where snow depths reached 96 cm on average, egg masses are deposited both above and beneath the snow on tree trunks and rarely on branches (Higashiura, 1989b).

In Canada, more than 40 species of birds are listed as predators of the gypsy moth (Benoit and Lachance, 1990). In eastern Canada, a recognized avian predator of gypsy moth eggs is the chickadee, Parus atricapillus (L.). Birds are also important larval predators, especially in low density innocuous gypsy moth populations but observations suggest that they find larger, hairy larvae unpalatable. Small insectivorous birds, such as warblers (Dendroicus spp.), will readily consume young gypsy moth instars. Such species of warblers migrate in large numbers through eastern North American deciduous forests in late spring when gypsy moth larvae are present (Whelan et al., 1989).

Like most birds that prey upon gypsy moth, small mammals are generalists. Shrews, moles and mice are most effective, although chipmunks and squirrels do provide some control (Benoit and Lachance, 1990). Vertebrate predators, especially the white-footed mouse, Peromyscus leucopus Raf. are the major source of late-larval and pupal mortality in the leaf litter. Larvae in the litter are less likely

to survive to the adult stage than those that are found in protected sites on tree trunks and branches (Campbell et al., 1975). However, Weseloh (1989b) attributes most of the predation of larvae in the litter to invertebrates, and predation in the tree to vertebrates, presumably small mammals (Weseloh, 1989a).

In low density populations (less than 50 egg masses/ha), larval mortality and predation rates on pupae are positively correlated with gypsy moth population densities. Predation by small mammals may be responsible for the regulation of low density gypsy moth populations. At high population densities, predation rates by small mammals are much lower. Increases in predation rates would probably be caused by a Type III functional response. Gypsy moth population density increases at an exponential rate. As prey density increases, the number eaten increases at an accelerating rate until a plateau is reached and no more can be consumed. This is a Type III functional response as described by Holling (1965). Predators that display a Type III functional response had either switched to eating gypsy moth from other prey species or else had learned to forage for gypsy moth as gypsy moth densities increase. However, since all predators are limited in the number of prey items that an individual can consume even a Type III response is asymptotic and therefore inversely density-dependent at high population densities (Elkinton and Liebhold, 1990).

Common invertebrate predators of the gypsy moth include a wide variety of insects and other arthropods. In eastern Canada, the black carpenter ant, Camponotus pennsylvanicus (De Geer) is a recognized insect predator of gypsy moth eggs (Benoit and Lachance, 1990). Ants also prey upon larvae and there can be a substantial number of ants

foraging on the forest floor where an encounter with a larva may occur every 20 minutes. These large numbers of interactions can make even low capture rates important (Weseloh, 1989a). In New England, the brown forest ant, Formica fusca, was found to be the predominant ant species that feeds upon gypsy moth larvae. When given a choice, F. fusca prefers first and second instars that had fed over first instars that had not. Unfortunately, unfed larvae are those found more often on the forest floor having just landed on the end of their silk lines (Weseloh, 1990). Larger larvae are more resistant to ant attack. Due to their greater size, strength and "hairiness", older larvae are more capable of repelling attacks from aggressive ants and are thus comparatively safe on the forest floor. Predation by ants in the litter may therefore be an important source of mortality for larvae in the early stages of development (Weseloh, 1989a).

Most imported parasitoids and predators have been introduced from Eurasia, India or Africa (Coulson et al., 1986). One important introduced predator is Calosoma sycophanta L. (Coleoptera: Carabidae). Importations of this beetle from Europe, India and Africa occurred between 1972 and 1977. C. sycophanta is an abundant, specific predator of gypsy moth larvae and pupae. Its life cycle is well-synchronized with that of the gypsy moth. Both larvae and adult beetles are predatory, with the latter feeding mostly on caterpillars and the former on pupae (Weseloh, 1988). Long-term observations in New Jersey suggest that the long-lived adults may help suppress low density gypsy moth populations and thus prevent outbreaks. Although C. sycophanta is widely distributed in New England, it has not spread as rapidly to the south and west as has the gypsy moth. Relocation

would speed up its dispersal and might improve existing gypsy moth control programs (Fuester, 1988).

The majority of parasitoids belong to two orders of insects, Diptera and Hymenoptera (Evans, 1984). Five dipteran and 16 hymenopteran families are known to parasitize L. dispar. There are more than one hundred known insect parasitoids of the gypsy moth in North America (Benoit and Lachance, 1990). Two of the most important egg parasitoids are Ooencyrtus kuvanae (Howard) (Hymenoptera: Encyrtidae), and Anastatus disparis (Ruschka) (Hymenoptera: Eupelmidae). They were imported beginning in 1972 from France and Morocco, and later from Africa, France, Japan and China (Coulson et al., 1986). A. disparis is rarely a significant source of mortality of the gypsy moth (Elkinton and Liebhold, 1990). A. disparis has not adapted readily to conditions in North America and has been slow to extend its range south and west, beyond the boundaries New York state. O. kuvanae is more successful at parasitizing the North American gypsy moth. Both species however have poor dispersal abilities, especially the flightless female A. disparis, which can only jump. Perhaps for these reasons O. kuvanae and A. disparis are unable to maintain North American gypsy moth populations at low densities (Schaefer et al., 1988; Elkinton and Liebhold, 1990).

Those parasitoids that utilize gypsy moth larvae have access to hosts for only about a couple of months every year. That parasitism, in combination with predation, can cause greater than 99% mortality within a generation was demonstrated by Gould et al. (1990) in experimentally created plots. Some larval parasitoids are specialists and their life cycles are closely synchronized with that of the gypsy

moth, while others are generalists that are able to utilize hosts other than the gypsy moth. One introduced specialist, Parasetigena silvestris (Robineau-Desvoidy) (Diptera: Tachinidae) is univoltine and oligophagous. P. silvestris oviposits large, macrotype eggs on the integument of gypsy moth larvae where they remain until the next moult. When parasitoid larvae hatch, they bore through the underside of the egg, through the hosts integument, and develop internally. Affected host larvae are severely debilitated and unable to pupate successfully. A new generation of P. silvestris emerges by the end of the fifth and sixth instar, just prior to gypsy moth pupation. Populations of P. silvestris are quite responsive to changes in densities of gypsy moth populations. The number of this species of parasitoid in a particular generation most likely depends on the number of hosts parasitized in the previous generation (Gould et al., 1990). On the other hand, generalist parasitoids may not be as dependent on the abundance of a particular prey species. Comsilura concinnata (Meigen) (Diptera: Tachinidae), another introduced larval parasitoid, is multivoltine and polyphagous. Gould and his co-workers (1990) demonstrated that C. concinnata is a major mortality agent during mid-larval stages of gypsy moth which acts in a positive density-dependent fashion. This tachinid showed a remarkable ability to locate and parasitize gypsy moth larvae in experimentally created plots. C. concinnata was able to respond quickly to increases in gypsy moth populations, either by migrating from surrounding areas, or by switching to the gypsy moth from alternate hosts. Larval parasitoids may have an important impact on the population dynamics of the gypsy moth. By suppressing local increases in the density of gypsy moths, prey levels might be maintained at levels where predation by small

mammals would be sufficient to prevent population increase (Gould et al., 1990).

Until lately, the most successful introduced gypsy moth pupal parasitoid was Brachymeria intermedia (Nees) (Hymenoptera: Chalcidae) (Fuester, 1988). There is evidence to suggest that it causes density dependent mortality however only at high gypsy moth densities. It appears to be very scarce in low density populations (Elkinton and Liebhold, 1990). B. intermedia also has difficulty attacking larger female pupae and older, harder pupae of five to nine days. A more recent introduction from India and Japan, Coccygomimus disparis (Hymenoptera: Ichneumonidae) may be an extremely useful addition to the parasitoid complex, particularly in association with B. intermedia. It is perhaps a better adapted predator than B. intermedia because it is able to develop on prepupae and pupae of all ages and it will readily attack female pupae (Fuester, 1988). C. disparis, however, is not specific to gypsy moths. This large ichneumonid also attacks hosts belonging to 14 lepidopterous families. The recent and successful establishment of C. disparis suggests that other parasitoids probably can be established. The principal factors contributing to the process of a successful introduction appear to include high numbers of parasitoids released, polyphagous habit of the parasitoid enabling it to use other hosts when gypsy moths are scarce and suitable overwintering behaviour or utilization of alternative hosts (Schaefer et al., 1989). However, parasite augmentation has not been shown to be an effective means of regulating gypsy moth populations in the northeastern United States (Blumenthal et al., 1981).

Pathogenic Microorganisms

There are advantages and disadvantages associated with the use of pathogens as biological control agents. The best pathogens are highly specific and have little or no effect on non-target species. They leave no chemical residue build-up in the environment. Some strains of pathogens are more effective than others. By placing a gene tag on a released strain, scientists are able to identify different strains in the field to assess the virulence of the released strain (Bidochka, 1993). Some disadvantages of the use of pathogens are that careful timing of spray applications is needed with those microbials which have an incubation period, the degree of host specificity may be too great so that certain life stages of the pest are virtually immune and each microbial has a moth population threshold below which the disease will not spread. Moth populations must be higher than this threshold if the microbial is to give effective control. The microbial may also lose its virulence in the production process and many microbials require damp climatic conditions for effective spread through the pest population (van Emden, 1974). These disadvantages have sometimes proved discouraging. However the potential of pathogens for pest control remains largely unrealized (Roberts et al., 1991).

Eighty-six pathogens, protozoans, bacteria, viruses and fungi, have been isolated from gypsy moths (Gerardi and Grimm, 1979). A small percentage of them are very successful. The most successful pathogens reside in the environment and are capable of activating quickly when conditions become optimum for development of an epizootic. Longer-lived stages of many entomopathogens reside in natural reservoirs such as in the soil or in protected cracks in tree bark (Hochberg, 1989) and become active in host populations during

specific combinations of host, pathogen and environmental conditions (Brown, 1987).

Microsporidians

Microsporidians are obligate intracellular protozoan parasites that induce chronic diseases in many organisms. In the gypsy moth, they infest muscles of the gut, silk glands, fat bodies, Malphigian tubules, muscles and gonads. Most infested larvae are killed in the last instars. Heavily infested females are sterile. Microsporidian infections cause a decrease in the number of ovarioles produced by the female moth. Microsporidians infest a new generation of host through its eggs. The infestation of newly laid gypsy moth eggs ranges from between 3% and 80%. Infested eggs have reduced resistance to stresses imposed by winter weather conditions (David *et al.*, 1989).

Gypsy moth larvae infested with a microsporidian species such as Vavraia spp. have heavily infected midguts. They produce spore-laden feces which other larvae may consume along with foliage. Other larvae, infested with a parasitic species, such as Nosema spp., are characterized by having heavily infected silk glands. Healthy larvae may ingest spores while following contaminated silk trails left by infested ones (Jeffords *et al.*, 1988).

Microsporidians are important pathogens of gypsy moth in Eurasia where they reportedly cause debilitating effects on populations and predispose larval populations to nuclear polyhedrosis virus (NPV) infection. Inoculative releases of five European isolates have been conducted in the Eastern United States and a species of Nosema was successfully transmitted among larvae. More research is currently being done with the aim of establishing this pathogen in North American gypsy moth populations (USDA, 1990).

Bacteria

Several bacterial pathogens associated with gypsy moth include Bacillus thuringiensis Berliner, commonly known as Bt, Streptococcus faecalis, Serratia marcescens, Serratia liquifaciens and several Pseudomonas spp. Infectious disease caused by bacteria usually does not exceed 15% of total mortality on average. However, there are times when bacterial infection may account for up to 60% within a given area. In 1972, half of the measured mortality in northern New Jersey was caused primarily by S. faecalis and Pseudomonas aeruginosa (Campbell, 1981). Occasionally disease caused by S. faecalis reaches epizootic proportions (Doane, 1970). Potential for causing widespread epizootics is probably limited by environmental factors. With the exception of Bt, the potential of most bacteria has not been studied extensively either because they are considered insignificant in natural population fluctuations or some property contraindicates them for use as microbial control agents (Campbell, 1981).

Bt was first recognized as a pathogen of silkworms in Japan and flour moths in Germany (Berliner, 1915). It is likely a worldwide and ubiquitous component of soil microbiota (Martin and Travers, 1989). Bt is actually a collective name for a complex of more than thirty known subspecies. A high level of specificity exists within each subspecies. Toxicity is specifically exhibited against Lepidoptera (subsp. kurstaki), Diptera (subsp. israelensis) or Coleoptera (subsp. tenebrionis) (van Frankenhuyzen, 1990). Bt is now widely used for control of gypsy moth, spruce budworm and several other defoliating insects in Canada. Insecticidal activity of this organism occurs when a parasporal protein body, often referred to as the crystal, is ingested by a susceptible insect. The crystal is converted by host digestive

enzymes to a toxic protein that destroys the cells lining the gut. If a lethal dose is ingested, the larva stops feeding and dies within a few days (van Frankenhuyzen, 1990).

Bt was first commercially available in the late 1930s in France as a product called Sporeine® but commercial production in North America did not begin until the early 1950s. Since then, the product has been refined to produce a narrow-spectrum insecticide that its proponents insist does not harm beneficial and non-target organisms (van Frankenhuyzen, 1990). Commercially available formulations of Bt include Foray 48B®, Thuricide®, Dipel® and SAN 415 SC 32LV® (USDA, 1990).

There are certain disadvantages associated with the use of Bt on gypsy moths. Production and application costs are high. Specific strains of this bacterium, although supposedly toxic only to a limited insect host range, may be harmful to benign lepidopterans. Also, host organisms exposed to it may develop resistance to scientifically-developed strains. Applications of Bt do not remain in or spread well throughout the forest. The field efficacy of Bt is dependent upon post-application occurrence of warm, dry weather conditions (Bennet, 1993). Since cool weather can slow larval feeding rates and rain can wash Bt off the foliage, adverse weather conditions can severely reduce the probability of gypsy moth larvae becoming infected.

Nuclear Polyhedrosis Virus (NPV)

Nuclear Polyhedrosis Virus (NPV) causes a disease that has been described since the early 1900s (Howard and Fiske, 1911). NPV currently occurs throughout the range of the gypsy moth and causes epizootics in high density populations (Elkinton and Liebhold, 1990). This disease was originally called "wilt" because infected cadavers

have a limp, collapsed appearance (Lewis, 1981). Bergold (1958) identified the causative agent of wilt disease as a nuclear polyhedrosis virus (NPV). The disease is now called nuclear polyhedrosis, a name derived from the fact that the virus causes polyhedra (see below) to be produced in the nucleus of cells of infected larvae (Lewis, 1981).

Gypsy moth NPV is selective for lymantriids (Lewis, 1981). Like other viruses, NPV is an intracellular parasite which requires living cells for multiplication. The virus replicates in the nuclei of susceptible larval cells where it produces two morphological forms, one occluded and the other non-occluded. The occluded form becomes embedded in a polyhedral body called a viral occlusion body. The polyhedral body protects the virus from environmental factors. The virus and its protective coat are collectively called a polyhedral inclusion body or PIB. The PIB stage is responsible for transmission of the disease in the insect population. When PIBs are ingested by a susceptible host, the proteinic matrix dissolves in the gut under the influence of an alkaline environment and proteolytic enzymes. The liberated virus initiates infection in midgut epithelial cells. The non-occluded form buds out of infected cells into the hemolymph and neighbouring cells and is responsible for dissemination of the virus within the host (Arif, 1991). Infected larvae die in about two weeks at which time infectious PIBs are released (Woods and Elkinton, 1987). These may be ingested by nearby hosts and thus the life cycle of the virus is ready to begin again.

Just before dying, infected larvae display a tendency to climb upward (Lewis, 1981). Although there are no external signs of viral presence, NPV-killed larvae are dark in colour and are usually found

hanging from the foliage or bark attached by their abdominal appendages in a characteristic inverted "V" shape. The cephalic and caudal portions of the body hang unattached (Hajek and Roberts, 1992). The fragile cuticle will rupture at the slightest touch, and emit a brown, foul-smelling liquid. The fluid contains large numbers of refractile PIBs, 1-10 μ m in diameter (Lewis, 1981). When the bodies of dead larvae rupture, abundant quantities of the virus are released into the environment. During an epizootic, massive numbers of PIBs are released from disintegrating cadavers of virally-killed larvae.

Both horizontal (within generations) and vertical (between generations) transmission of NPV have been investigated. The principle mechanism of horizontal transmission of NPV occurs when diseased neonates die in the first instar and become a source of NPV inoculum for surviving larvae of all stages. This gives rise to a characteristic bimodal temporal pattern of mortality. An early peak of mortality is evident due to early instar deaths. A second higher peak of mortality from NPV occurs in late instar larvae during the next wave of infection. Such a pattern of infection can lead to an epizootic late in the season under high host density conditions (Doane, 1969, 1970; Woods and Elkinton, 1987). Mechanisms of vertical transmission of NPV are not well understood. Three different modes of vertical transmission of virus have been suggested. Vertical transmission of NPV may occur from mother to eggs or egg masses, by environmental contamination of egg masses or by larval ingestion of inoculum persisting in the environment from the previous year (Murray and Elkinton, 1989).

Vertical transmission of NPV to eggs or egg masses occurs either through infection or external contamination of progeny by

moths. Eggs and subsequent larvae might become infected through transmission from sub-lethally-infected adults (transovarial transmission). The evidence for transovarial transmission is inconclusive. Shapiro and Robertson (1987) showed that infected females may be able to transmit infective NPV to some progeny in the laboratory. However, Murray and Elkinton (1989) were unable to demonstrate a similar pattern in the field. Transmission of NPV to eggs may be the result of environmental contamination of the female rather than internal infection. Doane (1976) and Podgwaite *et al.* (1981) suggested that NPV is transferred via PIBs on the mothers body to the surface of the egg (transovum transmission) during oviposition. Neonates may then become infected upon hatching if they ingest virus present on the egg mass surface (Doane 1969, 1970). Doane (1975) showed that setae deposited onto the egg mass from the abdomen of the female during oviposition are a good source of NPV inoculum for newly hatched larvae. Vertical transmission of NPV to eggs or egg masses is thought to occur only to a limited extent. It may have increased significance in years following an epizootic as a means of reintroducing low levels of virus into an increasing population and therefore initiating density-dependent spread of inoculum through the population and the environment (Murray and Elkinton, 1989).

Environmental contamination of egg masses occurs when females lay their eggs on a surface contaminated with viral PIBs. Murray and Elkinton (1990) demonstrated that higher mortality from NPV occurs in the field among larvae hatched from egg masses laid on artificially-infected bark substrates than among larvae hatched from egg masses laid on bleach-treated or untreated bark. Environmental contamination of egg masses is readily evident and is substantial in sites in which an

epizootic has occurred in the same generation (Woods and Elkinton, 1987). Environmental contamination of egg masses is also an important mode of transmission of virus from one generation to the next (Doane, 1970, 1975; Woods and Elkinton, 1987; Murray and Elkinton, 1989).

Vertical transmission may also occur when larvae ingest inoculum persisting in the environment from the previous generation. NPV can persist on bark for at least one year (Podgwaite *et al.*, 1979). First instars which traverse a virus-contaminated surface may become infected by ingesting contaminated food. Larvae may also become infected if they make oral contact with PIBs as they travel over pupal exuviae and cadavers. The importance of environmental persistence for vertical transmission of NPV across generations is not known (Lewis, 1981) but it may be critical for maintenance of the pathogen in low-density population in years following an epizootic (Doane, 1976; Podgwaite *et al.*, 1979; Weseloh and Andreadis, 1986)

Other processes, such as leaching by rain and passive transport by predators or parasitoids, may be important in dissemination of NPV throughout gypsy moth populations and the environment. Rainfall probably does not play an important role in leaching of NPV from the environment directly onto gypsy moth egg masses but it may be involved in spreading NPV throughout the environment prior to the oviposition period. Studies have shown that viable NPV can be found in the feces of gypsy moth avian and mammalian predators and thus it may be transmitted throughout the environment. Although the role of insect parasitoids in the transmission of the virus between egg masses has been questioned, it has not been examined in any great detail (Murray and Elkinton, 1989).

In 1978, Gypchek[®], a gypsy moth viral insecticide, was registered for commercial use by the Environmental Protection Agency for the USDA Forest Service (Lewis, 1981). Under optimal conditions, Gypchek[®] results in a 50-80% reduction in egg mass density, and defoliation of under 55% (Cunningham *et al.*, 1991). Gypchek[®] is reported to cause higher mortality (70-92%) than does naturally-occurring virus at the peak of an outbreak (27-55%) (Novotny, 1991). Treatment with Gypchek[®] eliminates caterpillars before they attain their maximum size and inflict the most damage on trees in the maximum defoliation phase (Woods and Elkinton, 1987). Another viral insecticide called Disparvirus[®] was developed at the Forest Pest Management Institute in Sault Ste. Marie. Reductions in egg masses per hectare (ha) after application of Disparvirus[®] ranged from 76 to 98% (Cunningham *et al.*, 1991). As with Gypchek[®], Disparvirus[®] is applied at a time when most larvae are in the first instar. Murray and Elkinton (1990) suggested an alternative time for spraying might have more beneficial effects. Since egg masses may become contaminated with NPV upon oviposition on contaminated substrates, application of NPV should rather be applied to trees in late summer before oviposition has begun.

However, infectious NPV is short-lived on foliage surfaces (Tanada, 1971) and several factors adversely affect viral activity including ultraviolet light and the chemistry and tannin content of foliage. NPV is harmed by solar radiation in the range of about 280-320nm. The activity of the virus is reduced by more than 50% in less than 48h in the presence of sunlight (McManus, 1988). The pH of the foliage that gypsy moth larvae consume affects their susceptibility to NPV. Foliage chemistry affects midgut pH, which in turn affects

dissolution rates of the occlusion bodies in which viral particles are embedded. Consequently, viral particles are not absorbed by the gut. The tannin content of foliage that gypsy moth larvae consume also affects their susceptibility to NPV. Some types of foliage, such as oak, contain high concentrations of tannins. Tannins bind with viral particles in the gut, inhibiting the passage of viral rods through the peritrophic membrane (Keating et al., 1988).

Fungi

There are about 700 species of fungi that infect insects (Bidochka, pers. comm.). Bacteria, viruses and fungi can all infect insects through the gut, but only fungi are also able to penetrate their host through spiracles and the surface of the integument. Therefore, fungi may be infectious independent of the feeding activity of the host. Fungi, the most common of all insect pathogens, play a key role in the natural regulation of many insect species (Ferron, 1978).

The entomogenous fungi do not occupy a definite systematic place but are instead distributed among all groups. A variety of modes of reproduction, particular to each systematic group, and a capacity to develop under different ecological conditions, produces a particular potential for action in the field of insect biological control. Deuteromycetes (Moniliales and Sphaeropsidales) and Zygomycetes (Entomophthorales) are two important groups. Foremost in importance among the Deuteromycetes is Beauveria bassiana (Balsamo) Vuillemin, a pathogen of many insect species, among them lepidopterans including the gypsy moth (Majchrowicz and Yendol, 1973). It overwinters in vegetable matter and is found in the soil (Brady, 1979). Other important members of the Deuteromycetes are Paecilomyces, Akanthomyces, Hirsutella and Metarhizium. The three entomogenous

species of Paecilomyces are P. farinosus (Holm ex S.F. Grey) Brown & Smith, P. fumosoroseus (Wize) Brown & Smith and P. tenuipes (Peck) Samson. Akanthomyces gracilis is pathogenic to caterpillars and members of Cercopidae in Ghana. Representatives of the genus Hirsutella are pathogenic to all systematic groups of insects Metarhizium is the agent of the green muscardine, a disease of flies. Members of the Entomophthorales are pathogenic to many groups of insects (Ferron, 1978).

Although many fungi have been reported as parasites of larval, pupal, and adult stages of insects, few are known to be associated with insect eggs in terrestrial environments. However, egg masses of gypsy moth should be especially sensitive to attack by fungal pathogens since the egg stage comprises about nine months of the life cycle. During this time, the egg masses are frequently exposed to cold, wet conditions which should favour fungal invasion. In addition, the eggs are usually laid on bark or under stones in close proximity to the soil. Bark and soil are more hospitable substrates for dormant propagules of fungi than are leaf surfaces or interior spaces within plants, where other species of herbivorous insects often lay eggs.

Dunbar et al. (1972) demonstrated the presence of P. farinosus in gypsy moth egg masses. An unidentified Candida was one of the more frequently isolated species obtained from field-collected gypsy moth egg masses (Majchrowicz and Yendol, 1973). Carroll (1987) isolated 20 species of fungi from gypsy moth eggs obtained in the field. Among them were the pathogenic species, B. bassiana, P. farinosus and Verticillium lecanii. Carroll also found these three pathogenic fungi in egg masses that were one year old. Survival of fungi in natural populations of gypsy moth would probably require that the fungus

persist for several years outside the host. Control of gypsy moth by a fungal egg parasite would require that the fungus persist at least through the summer months, between the time of egg hatch and new egg production (Carroll, 1987).

Many of the fungi which infect gypsy moth in North America today may have occurred in the environment prior to the introduction of this pest. They occurred on entomogenous substances or persisted as facultative saprotrophs in bark or litter. These and other inconspicuous microfungi should be expected wherever long-lived insect eggs remain dormant in warm, wet climates. There was a high overall level of mortality seen in egg masses sampled in Carroll's study (65%) which led him to suggest that entomogenous fungi may be deleterious to insects with lymantriid-like life cycles in which the eggs remain for a long period in dark damp places (Carroll, 1987).

Another field study conducted by Majchrowicz and Yendol (1973) was initiated because of a mycosis detected in some larval gypsy moth specimens. Relatively few fungal pathogens were found to attack larval or adult gypsy moths in this Pennsylvania study and none caused significant mortality. A greater proportion of specimens supporting fungal growth were obtained from larvae and pupae collected in areas that had been sprayed with Bt. A weakening of the host by bacterial infection may have facilitated a subsequent invasion by pathogenic fungi. B. bassiana and P. farinosus were isolated from over 80% of the fungi-containing specimens (Majchrowicz and Yendol, 1973). In 1990, B. bassiana and P. farinosus were isolated from gypsy moth larvae collected in southeastern Ontario (Welton, 1991).

Majchrowicz and Yendol (1973) noted no entomophthoralean infection of gypsy moth populations in their study. The

Entomophthorales includes over 100 organisms known to be specific pathogens of a variety of different insect hosts (Humber, 1984). Currently, characters used in the identification of entomophthoralean fungi include conidial size, host specificity, isoenzymic analysis (Soper et al., 1983), identification of antigens (Toriello et al., 1989) and polymorphisms in rDNA coding sequences. The latter are useful for discrimination of species and higher taxa but do not appear to be as useful in the delineation of lower taxa (Walsh et al., 1990). An important group within the Entomophthorales is Entomophaga spp. Members of Entomophaga spp. are obligately entomopathogenic (Humber, 1989). They attack important insect pests such as hemlock looper (Lambdina fiscellaria), spruce budworm (Choristoneura fumiferana), gypsy moth and several grasshopper species (Walsh et al., 1990). Foremost among this group of entomopathogens is Entomophaga aulicae (Reichardt in Bail) Humber.

At present, E. aulicae is considered a species complex that infects only Lepidoptera and has been found throughout the northern hemisphere. Members infect hosts in nine lepidopteran families (Speare and Colley, 1912; MacLeod and Muller-Kogler, 1973), but mainly in four families of the Noctuoidea. These are, the Arctiidae, Lymantriidae, Noctuidae, and Notodontidae. However, E. aulicae infections have also been reported from the Geometridae, Lasiocampidae, Tortricidae, Sphingidae, and Nymphalidae (Speare and Colley, 1912; MacLeod and Muller-Kogler, 1973).

Fungal morphology alone cannot distinguish members of the E. aulicae complex and not enough is known of development and pathology for these characteristics to be used definitively for species identification. The clearest criterion separating this species from

other members of the complex is isozyme composition. Restriction fragment length polymorphisms (RFLPs) have been used to differentiate fungal isolates within and between species. Existence of species-specific RFLP patterns has been shown among 24 different Entomophaga isolates (Hajek et al., 1990a). However the complex is poorly described at present and contains only two species, E. aulicae, for which type material is unavailable (Hajek et al., 1991) and the gypsy moth pathogen, Entomophaga maimaiga which was originally described by Soper et al., (1988) from L. dispar in Japan.

The Gypsy Moth Fungus, Entomophaga maimaiga

The fungus, Entomophaga maimaiga Humber, Shimazu and Soper (Entomophthorales: Entomophthoraceae) is a specific and highly virulent fungal pathogen of the gypsy moth (Andreadis and Weseloh, 1990). In Japan, where it is one of the most important natural mortality agents of the gypsy moth, E. maimaiga periodically causes extensive epizootics that are capable of completely destroying outbreak populations of L. dispar (Aoki, 1974; Shimazu and Soper, 1986; Shimazu et al., 1987). E. maimaiga can have a large but variable impact on gypsy moth populations and was responsible for extensive epizootics of gypsy moth in the north-eastern parts of the United States in 1989 (Andreadis and Weseloh, 1990). As a result, much interest has been generated in assessing the potential of E. maimaiga as a biological control agent of gypsy moth in North America.

Introduction and Spread in North America

In the early part of this century, Speare and Colley (1912) made the chance discovery of an entomophthoralean fungus on some dead Japanese gypsy moth larvae in Massachusetts. The newly discovered fungus resembled E. aulicae (previously known as Entomophthora

aulicae), an organism which they had used successfully in the control of populations of another lymantriid pest, the brown-tailed moth, Euproctis chrysorrhoea. Despite its close resemblance to E. aulicae, Speare and Colley identified the Japanese fungus as a separate entity and referred to it as the "gypsy fungus" because of its close association with its host, the gypsy moth (Speare and Colley, 1912). Soper et al. (1988) believe that Speare and Colley's "gypsy fungus" was Entomophaga maimaiga.

In 1910-11, Speare and Colley (1912) obtained some "gypsy fungus" from Japan and released it in several locations near Boston. Their experimental releases were terminated once their fungal cultures became depleted. Methods that would have enabled them to grow the fungus in vitro had not yet been developed and their stocks of gypsy moth larvae either were used up or were decimated by an outbreak of NPV. The released fungus was not recovered from the field and, therefore, their introduction was considered a failure (Soper et al., 1988).

In 1974, a series of epizootics in Japan broke out with reported mortality rates of up to 99% amongst gypsy moth populations (Aoki, 1974). These epizootics were attributed to an entomophthoralean fungus believed to be the same species as the one that had been released around Boston by Speare and Colley (Soper et al., 1988). Based on these data from Japan, a second attempt was made to introduce the fungus into North America. In 1985 and 1986, larvae infected with E. maimaiga were imported from Japan and released into Allegheny State Park, New York, and Shenandoah National Park, Virginia (Andreadis and Weseloh, 1990). However, like the initial introduction, this one was also deemed unsuccessful. Incompatibility

between pathogen and host due to strain differences between Japanese and North American gypsy moths, and improper introduction methodology were thought to have been partially responsible for the lack of success (Hajek and Roberts, 1991).

In 1989, large numbers of dead and dying gypsy moth larvae were found throughout many forested and residential areas of the northeastern United States. The disease was first noticed in late spring when most larvae were in the fourth stadium. Microscopic examination of these larvae showed the presence of a fungal pathogen morphologically identical to E. maimaiga. This was the first reported occurrence of this fungus in North American gypsy moths (Andreadis and Weseloh, 1990). Another major fungal epizootic caused by E. maimaiga occurred in 1990 (Elkinton et al., 1991).

There are several theories to explain this sudden appearance of E. maimaiga in North America. The fungus may have remained dormant or been maintained at very low levels since its initial release in the early 1900s, and may have been spreading slowly throughout the intervening years. It may have gone undetected because environmental conditions, most notably rainfall, were not adequate to initiate large epizootics (Andreadis and Weseloh, 1990). It may also have increased in virulence over the years and, once a high level of virulence had been achieved, it may then have actively spread throughout local gypsy moth populations (Hajek and Roberts, 1992). It is also possible that fungal-induced epizootics had actually occurred earlier in the century but had been mistakenly attributed to other pathogens such as the gypsy moth nuclear polyhedrosis virus. On the other hand, its present abundance may be attributed to more recent accidental introductions. Egg masses are capable of harbouring fungal resting spores (Aoki et al.,

1976). E. maimaiga may have been inadvertently introduced very recently via contaminated egg masses from Japan, in conjunction with experimental egg parasitoid releases (Andreadis and Weseloh, 1990). The exact mechanism responsible for today's wide distribution of E. maimaiga in North America is unknown but the geographical distribution of the fungus in 1989 suggested that its origin was in New England where the initial releases occurred in the early 1900s (Hajek et al., 1990a). Its prevalence and wide distribution also suggest that it has been established for some time (Hajek et al., 1990a). In 1989, it was found in Massachusetts, Connecticut, New Hampshire, Vermont, eastern New York, northeastern Pennsylvania and New Jersey. In 1990, E. maimaiga was reported from at least ten states in the northeastern United States (Elkinton et al., 1991). More recently, it was reported from Belleville, Ontario, in Canada (Welton, pers. comm.) (Figure 5).

Mode of Infection

The three main stages in the life cycle of E. maimaiga, the overwintering, infective and vegetative stages, are represented by resting spores, by conidia and by protoplasts and hyphae respectively. The life cycle of E. maimaiga, illustrated in Figure 6, is described below.

Resting spores comprise the overwintering stage in the life cycle of the fungus. Resting spores (20-40µm in diameter) are hyaline to light-yellow, spherical in shape and have a bilayer wall (averaging ca. 2.0µm thick) (Figure 7). Resting spores develop from azygospores which themselves are formed by budding from fungal hyphal bodies within the host. Infections in the spring are initiated when resting spores in the soil and leaf litter begin to germinate and release

Figure 5. Distribution of sites across the northeastern United States and southern Canada where E. maimaiga was recovered [●] and was not recovered [○] from cadavers of gypsy moth larvae in 1990 (from Hajek et al., 1990a; Welton,1991).

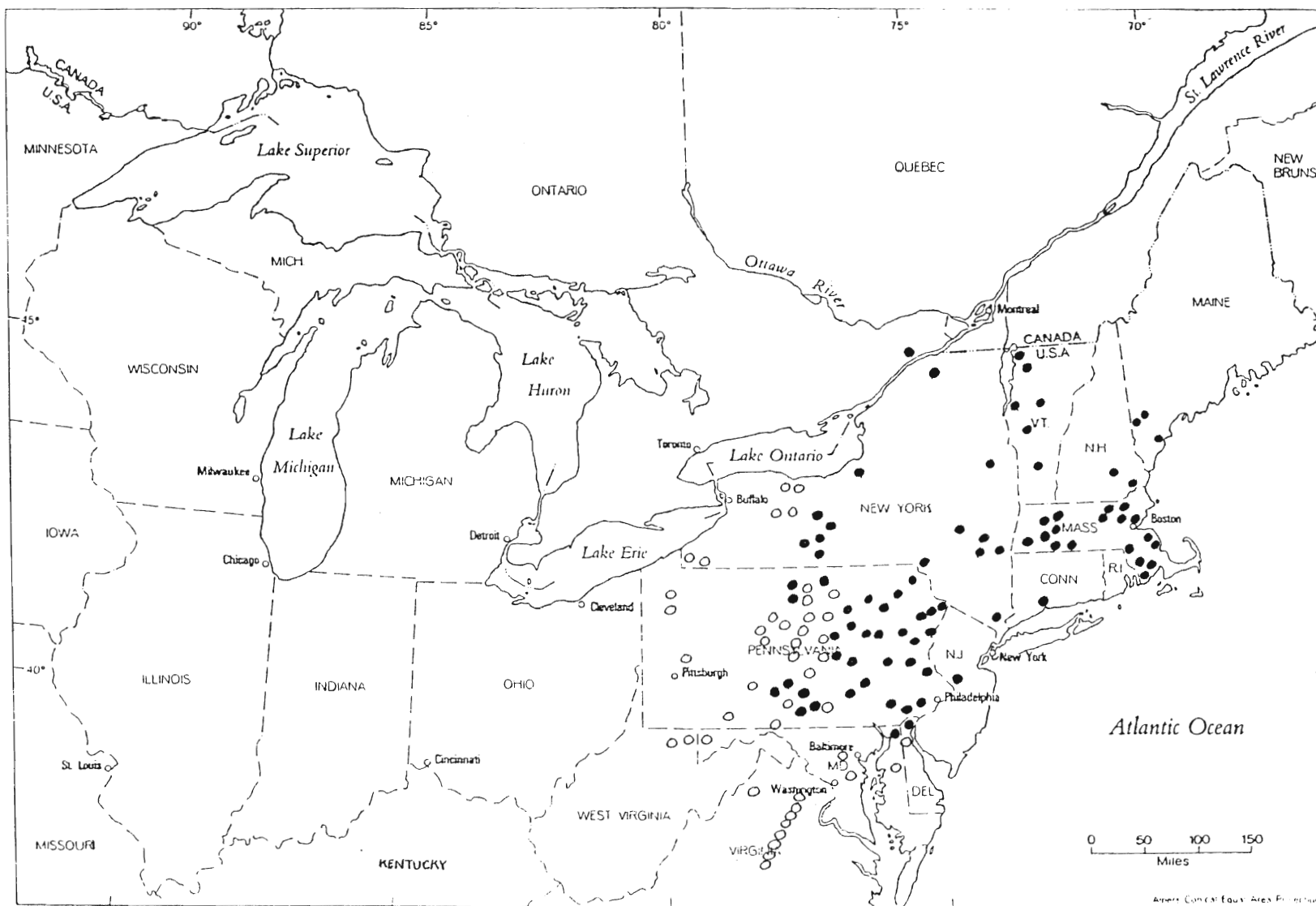
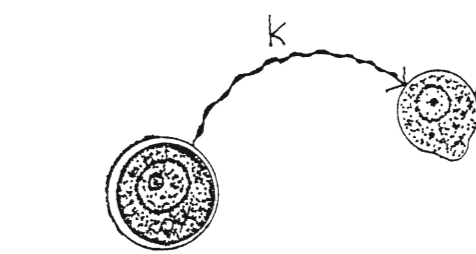
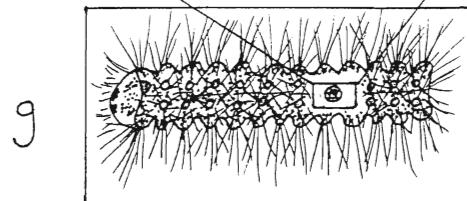
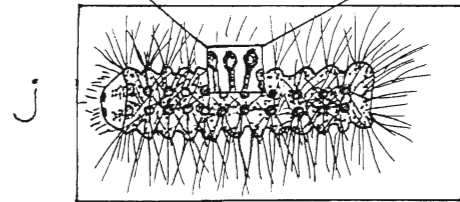
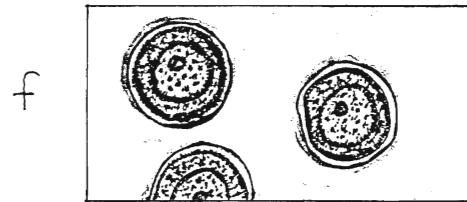
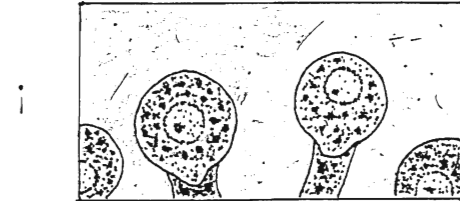
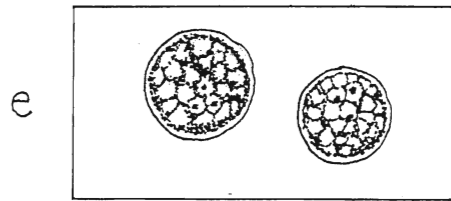
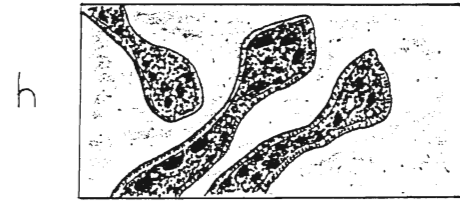
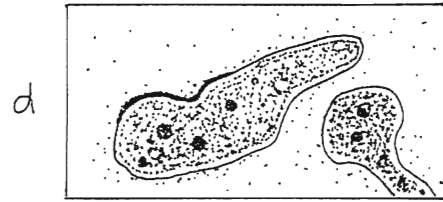
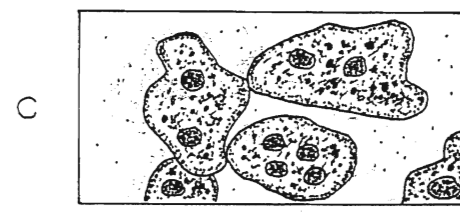
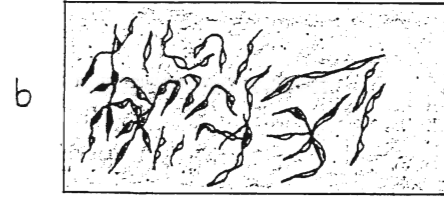
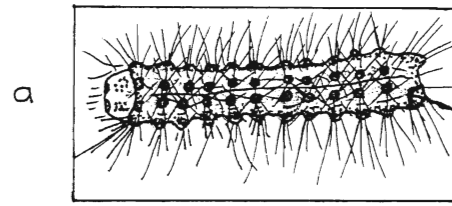


Figure 6.

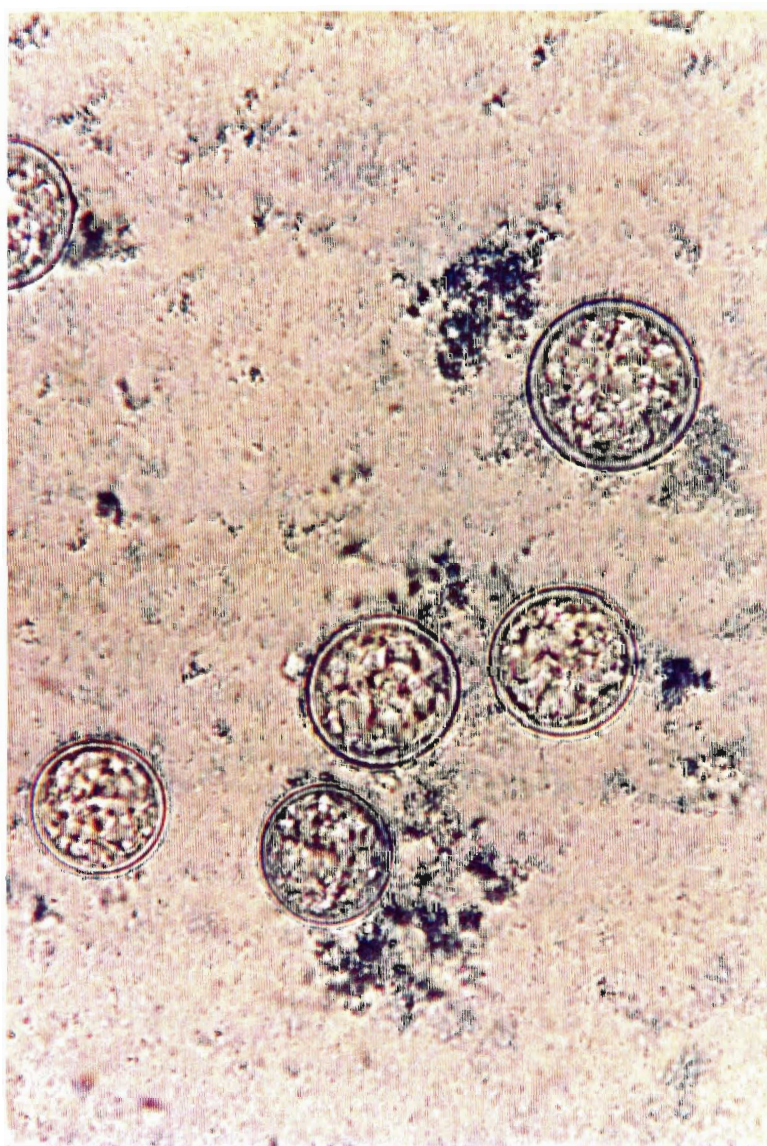
Life cycle stages of E. maimaiga.

(a) penetration of germ tube through cuticle of larva and subsequent release of protoplasts into hemolymph. (b) proliferation of protoplasts in insect hemolymph. (c) formation of hyphal bodies. (d) movement of cellular contents of hyphal bodies into bud-like structures. (e) immature azygospores. (f) resting spores. (g) overwintering resting spores in larval cadaver. (h) conidiogenous cells. (i) conidia situated on terminal end of conidiophores. (j) sporulation of conidia. (k) germination of a resting spore produces a conidium. (l) passage of a conidium on air currents and subsequent contact with a gypsy moth larva (from Soper et al., 1988).



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Figure 7. Resting spores of E. maimaiga (x400).

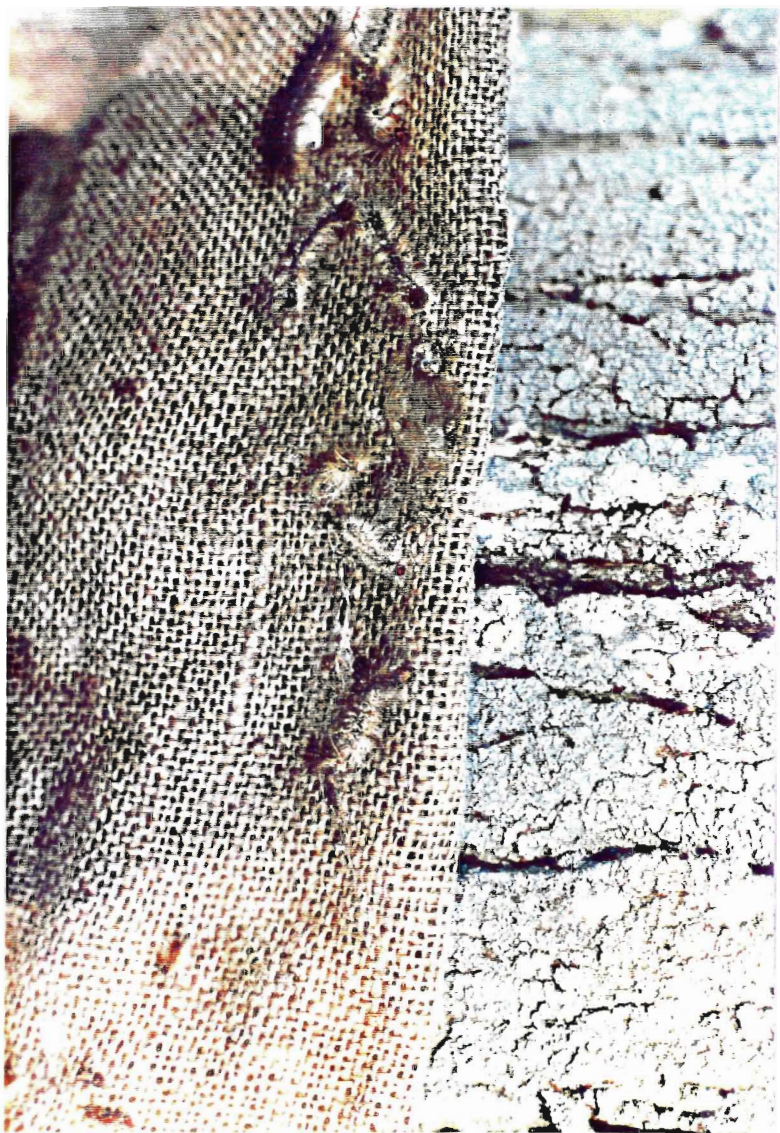


conidia (Andreadis and Weseloh, 1990). It is unclear exactly when germination occurs in the field or what the cues are which lead to its initiation. Conidia are the infective stage of the fungus. Conidia (ca. 26 x 21µm) are colourless to light yellowish- or pinkish-tan, pear-shaped (Soper et al., 1988) and have adhesive properties which enable them to adhere to nearby host bodies. If conidia fail to contact a host, they may produce secondary conidia. Secondary conidia are similar in shape but smaller in size (Andreadis and Weseloh, 1990). Once a conidium comes in contact with a host, it produces a germ tube which penetrates the host integument. After conidia make contact with the host cuticle, less than 24h is required for them to penetrate the host and start infection (Hajek, unpublished data). After the germ tube has penetrated the host integument, small wall-less cells called protoplasts are released into the host hemocoel. This protoplasmic form of many entomophthoralean fungi might be a means by which the fungus escapes detection by the host's defence system. Hemocytes of natural hosts will encapsulate walled cells of E. aulicae, for example, but do not recognize its wall-less protoplasts (Dunphy and Nolan, 1982). Protoplasts proliferate in the hemolymph. Three days after inoculation with E. maimaiga, protoplast proliferation may be great enough to seriously alter larval metabolic processes and affect normal growth (Hajek, 1989). Protoplasts form bead-like chains of cells in host hemolymph, round up and eventually develop cell walls late in development (Soper et al., 1988). The development of hyphal bodies throughout host tissues is noted as the disease progresses. Hyphal bodies are unicellular, irregular to sac-like in shape and multinucleate. In the laboratory, larvae infected with E. maimaiga show a decrease in food consumption (Hajek, 1989) and die within five

to six days post-infection (Soper et al., 1988). This mode of infection, the production of infective conidia from overwintered resting spores, is termed primary transmission, and it is characteristic of fungal mortality in early instars at the beginning of the season. After host death, the hyphae produce simple, unbranched conidiophores which grow out through the integument and form a velvet-like coating over the cadaver (Figure 8) (Dunphy and Nolan, 1982). These conidiophores then sporulate to produce conidia which may infect other susceptible larvae nearby. Thus, older instars are mainly infected through conidia produced from sporulating conspecific cadavers (secondary transmission). It has been demonstrated in the laboratory that each larva produces 1×10^5 to more than 1×10^6 infective conidia. This high rate of emission can cause a heavy epizootic in older larvae later in the season (Shimazu et al., 1987). Secondary transmission via conidia produced by diseased larvae is probably necessary to amplify the initial infection if an epizootic is to occur (Weseloh and Andreadis, 1992a). Progressive seasonal increases in infection levels may also be associated with diurnal wandering behaviour exhibited by late instar larvae (Weseloh, 1989b). This behaviour would increase larval exposure to germinating resting spores in soil or bark reservoirs (Hajek and Roberts, 1991).

Infection with E. maimaiga may produce another type of cadaver, one producing resting spores rather than conidia. As the season progresses and as the larvae become older and larger, resting spore production occurs with increasing frequency in infected fifth and sixth instar cadavers and eventually dominates over conidial production. A laboratory study showed that more conidia than resting spores are produced in cadavers incubated under dry conditions after death. The

Figure 8. Sporulating conidia on gypsy moth cadavers.



moisture content of the body before death may, therefore, be an important factor in determining whether conidia or resting spores are produced. A younger larva has a smaller body than an older one but the surface area per weight of the younger larva is larger. Younger larvae may lose internal water faster than older ones and thus produce the drier conditions optimal for conidial production (Shimazu, 1987).

After host death, E. maimaiga may be easily visible on some cadavers. Conidia were visible on setae of 22% of cadavers killed by E. maimaiga in a field study performed by Hajek and Roberts (1992). A correct field diagnosis may also be possible, even if conidia are not present, based on the appearance and position of the cadaver. It is important to be able to distinguish fungal-killed larvae from those killed by NPV which look similar. Insects infected with NPV often climb to elevated locations before dying. However, gypsy moth larvae infected with E. maimaiga do not become geonegative prior to death (Hajek and Soper, 1991). Unlike those killed by the virus, fungal-infected caterpillars are found attached only to the lower portions of tree trunks, and not on small upper branches and foliage (Hajek and Soper, 1991). Many cadavers hang vertically with their heads directed downward (Aoki, 1974; Andreadis and Weseloh, 1990) and with the prolegs at a 90° angle to the longitudinal axis of the body (Figure 9) (Hajek and Roberts, 1992). Others are typically found attached by their prolegs to the lower portions of tree trunks with their heads pointed downward in a position similar to those killed by NPV. However, those larvae recently killed by the fungus, though soft-bodied and flaccid, are much more resilient and less easily ruptured than those killed by NPV (Shimazu and Soper, 1986).

Figure 9. Characteristic form of a gypsy moth larva infected with E. maimaiga.



Since fungal-filled cadavers remain attached to tree trunks for several days, or even longer, many resting spores may be leached onto the tree bark. These spores might then serve as inoculum for the next season, when they would germinate and infect young larvae moving up and down tree trunks (Hajek and Soper, 1992). Resting spores can also be found in the leaf litter where first instars come in contact with them when they fall to the ground after ballooning. The fungus is then spread by the infected larvae as they disperse into the canopy and subsequently die (Weseloh and Andreadis, 1992a). Resting spores in the leaf litter appear to germinate more successfully than those that remain all winter in cadavers on tree trunks, probably because the former are better protected from adverse environmental conditions (Weseloh and Andreadis, 1992a). It has been suggested that resting spore germination may occur throughout the summer. Hajek and Roberts (1991) speculated that large number of infections of older larvae may be due to primary rather than to secondary transmission. Since late-stage larvae rest in leaf litter during daylight hours (Leonard, 1970), they would be likely to become infected because of their close association with germinating resting spores in the soil. However, experiments monitoring infection levels in caged larvae in the field indicated that most infections in late spring were initiated by conidia produced by infected larvae and not by germinating resting spores (Weseloh and Andreadis, 1992b).

Resting spores can be abundant in the immediate environment the year after an epizootic (Shimazu et al., 1987; Hajek and Roberts, 1991). In Japan resting spores were found to be viable for less than two years (Weseloh and Andreadis, 1992b). If the lifespan of resting spores is indeed less than two years, the fungus must alternate its

generations every year in order to revitalize the stock of resting spores in environmental reservoirs. For the disease to prevail in new host populations of gypsy moth, migration of infected larvae may be a prerequisite. Once they have died, conidia from these infected migrant larvae will sporulate and contaminate other gypsy moths in hitherto uncontaminated populations (Shimazu et al., 1987).

Possible mechanisms of dispersal are as follows: (1) expulsion of conidia from germinating resting spores or from infected cadavers (Weseloh and Andreadis, 1992b), (2) dispersal of infected late instar larvae (Hajek and Roberts, 1991), and/or (3) transport by vector agents such as predators or scavengers which have fed on diseased insects or cadavers (Gugani and Okafor, 1980).

In the case of some fungi, such as B. bassiana, an epizootic may be initiated by the dispersal of spores which are small and easily blown away by the wind. Studies have documented the presence of five entomophthoraleans in the air currents (Wilding, 1970). However, conidia and resting spores of Entomophaga are too large to be carried long distances by the wind. Roberts and Humber (1981) hypothesized that attachment of cadavers of fungus-killed insects in specific, usually elevated locations allows for better spore dispersal. However, in the case of E. maimaiga, Weseloh and Andreadis (1992a) observed that there is no evidence that height of exposure was important. Resting spores may also be dispersed to other locations on the bodies of animals that contact them on the tree or in the leaf litter. Different life stages of the fungus may also be carried to new areas by the live host itself. In one field study, it was estimated that the fungus spread about 350m from a release site in one year (Hajek, pers. comm.). Two years after the initial release, the fungus had spread to a

distance greater than one kilometre from the point of release (Witcosky, 1993).

Specificity, Pathogenicity and Virulence

All stages of insect development are susceptible to mycosis, but all insect species are not susceptible to the same fungi (Ferron, 1978). Gypsy moth larvae show susceptibility only to isolates of E. maimaiga which originated from Japanese gypsy moths and they have never been infected successfully by any North American isolates of Entomophaga spp. from other lepidopteran hosts (Soper et al., 1988).

The Entomophthorales, the order to which E. maimaiga belongs, includes species with host ranges confined to one insect family or genus, or even to a few species (Soper et al., 1988). E. maimaiga is a virulent pathogen of gypsy moth and another lymantriid, the Douglas-fir tussock moth, Orgyia pseudotsugata, and a poor mortality agent of other lepidopterans (Soper et al., 1988). Laboratory studies with one isolate, ARSEF (Agricultural Research Service collection of Entomopathogenic Fungi) 1400, showed it to be only weakly pathogenic to lymantriids other than the gypsy moth (Soper et al., 1988). E. maimaiga can successfully infected larvae in the suborders Noctuoidea, Bombycoidea, and Geometroides. In all families, however, except the Lymantriidae, infection was not uniform and infection levels were low (Hajek and Butler, 1993).

Different isolates within the same species can vary in pathogenicity and virulence (e.g. Holdom et al., 1988). For example, L. dispar larvae infected with ARSEF 1392 and ARSEF 1400 took between five and six days to die while those infected with ARSEF 1391 took between seven and ten days. However, the incubation period until death may not be representative of natural circumstances since larvae

were infected in the laboratory through intracoelomic injection and maintained at constant temperatures (Soper et al., 1988).

Repeatedly subculturing E. maimaiga in the laboratory results in aberrant morphology of protoplasts in liquid media, decreased virulence and diminished mortality. There is a shift in morphology of vegetative protoplasts from small fusoid cells and bead-like chains of wall-less cells to enlarged spherical protoplasts. The absolute length of time that protoplasts are in axenic culture does not influence pathogenicity while the number of times cultures are sub-cultured can change fungal virulence. Incubation time of the disease increased significantly after 15 in vitro passages. Aliquots subcultured nine times caused larval death more quickly than those subcultured 25 times. Mortality occurred more quickly after one to two passages than after 12-15 passages. After 50 passages, only 20% of infected larvae died. A fungal isolate, weakened by excessive sub-culturing, can have its virulence restored if it is passed through an insect's body before again being recultured (Hajek et al., 1990b).

Environmental Requirements

Factors that influence transmission of pathogens from diseased to healthy individuals regulate a critical step in insect disease dynamics. Generally, fungal entomopathogens kill their hosts and then develop into a life stage which can live outside of hosts, before they become able to produce spores and potentially cause renewed infections (Roberts and Humber, 1981). In order to produce and discharge spores, environmental conditions must be favourable to the fungus after death of the host. Moisture is probably the most important environmental factor although others, such as temperature

and light, may also affect pathogen activity (Roberts and Campbell, 1977).

Conidial germination requires high humidity with almost 100% relative humidity needed for infection to develop (Shimazu and Soper, 1986). Laboratory experiments indicated that conidia germinate only in the presence of free water, with no conidia germinating at humidities lower than 70% (Hajek *et al.*, 1990c). High moisture conditions from one to three hours prior to conidial release seem to be most critical. This is consistent with the assumption that host cadavers must rehydrate to optimal levels before producing and releasing conidia (Hajek and Soper, 1992). Conidia are discharged in abundance under saturated conditions, but discharge lessens with decreasing humidity (Hajek *et al.*, 1990a). Cadavers have been observed to sporulate, cease sporulation, and later sporulate again, with much reduced numbers of conidia. The ability of fungal entomopathogens to undergo interruptions in spore release and then later to resume sporulating has been reported from several host-fungal pathogen systems. Hypera postica (Gyllenhal), killed by an Erynia sp., was observed under conditions of varying humidity. Although conidial discharge was depressed during periods of lowered relative humidity, the fungus recovered when exposed again to saturated conditions. However, as conditions became more severe, the ability of this fungus to recover declined (Millstein *et al.*, 1983). Tyrrell (1988) discovered that if dehydrated cadavers of Choristoneura fumiferana (Clemens), killed by E. aulicae, were rehydrated, they were able to produce spores, albeit at lower levels, 84 days after death of the larvae. It was necessary to soak the cadavers in water. They were

not able to produce spores if they were merely exposed to conditions of 100% relative humidity.

Water, either in liquid or vapour form, is essential for the germination of spores of most fungi, and thus high atmospheric humidities favour development of epizootic mycoses. Under conditions of 100% relative humidity, multiplication of inoculum results in a heavier contamination of healthy insects which in turn favours epizootic development. Large amounts of rainfall early in the season may be critical for development of the fungus. Moisture levels in the environment stimulate germination of resting spores. The prevalence of E. maimaiga in irrigated and non-irrigated areas was examined in the field. Prevalence of the fungus was slightly higher in forest-collected larvae from irrigated areas and significantly higher in caged larvae from irrigated areas (Weseloh and Andreadis, 1992a).

In the three years following the discovery of the fungus in North America, the last year, 1991, was the only one in which levels of spring rainfall were not above average. Although disease prevalence was lower in 1991 than in the previous two years, E. maimaiga was still active. Therefore, it seems that even under suboptimal conditions, the fungus can still germinate and infect a large proportion of the gypsy moth population (Weseloh and Andreadis, 1992b). Ferron (1978) suggested that infection of insects may occur independently of environmental humidity. The boundary layer of the insect integument may facilitate spore germination even if the atmosphere is practically dehydrated.

The rapidity of mycelial development, and therefore the speed of infection, depends on temperature. For most fungi, optimum temperature values fall between 20°C and 30°C with limits between

5°C and 35°C (Ferron, 1978). For E. maimaiga, approximately 80 degree days are needed for fungus development (Weseloh and Andreadis, 1992). Conidial formation can be influenced by temperature. Conidial discharge is initiated more rapidly in the laboratory at 20°C than at 15°C or 25°C. No sporulation occurred at 30°C, while at 2°C sporulation occurred after one month (Hajek et al., 1990a). Conidial germination on the host is also affected by temperature. However, spore type formed within host cadavers is not temperature-dependent although temperature influences spore development in several other Entomophthoraceous fungi (Shimazu and Soper, 1986). The optimum temperature for development of fungus is not necessarily the same as that for development of the disease in the moth host (Ferron, 1978). For E. maimaiga, the optimum temperature for infection to occur is 15°C while time to death of the moth is shortest at 20°C (Shimazu and Soper, 1986).

The influence of temperature on the host insect must be taken into consideration. Very short periods between moults as a consequence of higher temperatures may reduce, for example, the duration of an instar. As a consequence the number of fungal penetration events may be reduced (Ferron, 1978).

Germination, conidial production and discharge of conidia are all affected by photoperiod. Germination occurs much more rapidly in dark laboratory conditions than in light (Hajek et al., 1990a), and cadavers produce maximal numbers of conidia in the scotophase. Sporulation is maximal during the night and early morning hours when it is dark, when temperatures are usually lower and humidity is higher. Whether E. maimaiga is responding only to temperature and humidity is unknown (Hajek and Soper, 1992). Aoki (1981) found that a closely

related fungus (Entomophthora "grylli" type), infecting Mamestra brassicae L., also demonstrates a marked tendency to sporulate more during the night.

Interactions with NPV

The two pathogens, E. maimaiga and NPV, have had a closely related history in North America since their introduction at the turn of the century. For many years prior to the discovery of the fungus in 1989, gypsy moth deaths may have been wrongly diagnosed as viroses rather than as mycoses. In the period between 1911 and 1932, June rainfall was strongly associated with gypsy moth population decline. Later studies performed in 1958-1963 showed that disease incidence was higher on wetter sites (Campbell, 1981). Such population declines were attributed to epizootics of NPV leading to the belief that wet years promoted epizootics of the virus (Hajek et al., 1990b). However, more recent research suggests that increased rainfall is associated with a prevalence of fungal-caused mortality. Confusion regarding the identity of the causative agent probably arose because both pathogens produce superficially similar-looking cadavers. Cadavers killed by one may easily have been mistaken for those killed by the other.

It is difficult to differentiate the impact of E. maimaiga from that of NPV on gypsy moth populations because both may exist together in the same host. However, it is believed that only one pathogen is active in a population at any given time. In other words, when the prevalence of NPV is high that of the fungus is low and vice versa (Andreadis and Weseloh, 1990). During the first officially-recorded fungal epizootic in North America in 1989, gypsy moth infection with NPV was rare (Andreadis and Weseloh, 1990). In contrast, disease prevalence in Allegheny forest in 1990 due to E.

maimaiga and NPV was $38.1 \pm 9.3\%$ and $54.6 \pm 6.0\%$ respectively. Few cadavers had evidence of both fungal and viral infection (Hajek and Roberts, 1992). In those cadavers having signs of both pathogens, it was impossible to discern which pathogen actually caused death. Pathogen development rate is influenced by temperature and concentration of inocula. E. maimaiga and NPV have very different developmental rates at different temperatures (Hajek, unpublished data). Concentrations of inocula of these two pathogens causing infection in the field are unknown. In some cases, these two pathogens do not prevent development of each other *in vivo*. The percentage of co-infections may have been underestimated because, if E. maimaiga-infected larvae subsequently became infected with NPV, they might have died before PIBs were formed and thus NPV would not have been detected microscopically (Hajek and Roberts, 1992).

Factors that influence the success of one pathogen over the other are of interest in the field of biological control of the gypsy moth. These factors include dispersal ability, humidity, and host population densities. NPV occurs throughout the range of the gypsy moth (Hajek and Roberts, 1992) while the range of the fungus seems more limited. The fungus may not be as efficient at dispersing as the virus. Or perhaps its entry to North America was more recent and, given time, its range will extend as greatly as the virus. Humidity has little influence on the development of NPV (Woods and Elkinton, 1987) unlike its great influence on the development of E. maimaiga. E. maimaiga seems to be particularly effective during years when high precipitation occurs (Weseloh and Andreadis, 1992a). Optimal host density levels for each pathogen may differ. NPV is most active in high density gypsy moth populations (Elkinton and Liebhold, 1990), but

the optimal host population level for development of E. maimaiga is unknown.

Influence of Host Population Density

One factor that may affect pathogen effectiveness is host population density. A density-dependent effect has been observed in spruce budworm, C. fumiferana, populations infected with other entomophthorales (Vandenberg and Soper, 1978). Such an effect has not been definitively shown to occur in gypsy moth populations. The relationship between E. maimaiga and gypsy moth population density is not clear and few investigations have been performed to elucidate it. In Japan, outbreaks of E. maimaiga occur at the terminal stages of an outbreak of gypsy moth when population densities are extremely high (Shimazu et al., 1987). In North America, the first epizootic of E. maimaiga occurred when gypsy moth populations were just beginning to increase after seven or eight years at low densities (Hajek et al., 1990a). The highest prevalence of the disease appeared to be in southwestern Connecticut where gypsy moth population densities were highest. However, the fungus was also found along the leading eastern edges of the infestation where gypsy moth populations were very low (less than 100 larvae/ha) (Andreadis and Weseloh, 1990). Elkinton et al., (1991) reported that mortality of gypsy moth larvae was not correlated with population density during the first fungal epizootic. The proportion of dead larvae was unrelated to density of gypsy moth as measured by the \log_{10} of total counts of larvae and pupae (alive and dead) under and below burlap bands placed in field plots. With stepwise multiple regression, rainfall, but not density, was detected as a significant correlate of proportion of gypsy moths dying as a result of infection with E. maimaiga (Elkinton et al., 1991). Hajek et

al. (1990a) performed an intensive study on four field plots of varying densities and their data indicated that no relationship existed between E. maimaiga-induced mortality and gypsy moth population density. Mortalities from E. maimaiga of greater than 50% were recorded even in low density plots. Hajek and Roberts (1991) hypothesized that E. maimaiga may help maintain gypsy moth populations in the low density endemic phase. In this respect, the gypsy fungus may be superior to other entomopathogens, such as NPV, which operate most effectively at high density host populations (Doane, 1970). High mortality is most desirable prior to the development of high density, highly destructive populations of gypsy moth.

Weseloh and Andreadis (1992a) concluded that E. maimaiga seems to be an effective natural enemy of the gypsy moth during years when host larvae are abundant. Infection rates were correlated with gypsy moth density and tree composition. Plots that had a higher composition of oak, and therefore a higher gypsy moth population density, had a higher infection rate. Gypsy moth density and percent oak composition are undoubtedly correlated because oak leaves are the primary food for gypsy moths. The greater impact of the fungus at higher densities of the host is probably because of the greater frequency of encounters of the pathogen with the host (Weseloh and Andreadis, 1992a).

Artificial Introduction into Host Populations

E. maimaiga does not occur in all areas currently colonized by its host (Hajek and Roberts, 1991) which suggests that it may not naturally spread as quickly as the gypsy moth. Investigation of methods expanding the range of this pathogen, by introducing it to novel areas using different fungal life stages, is therefore important.

There are four ways in which field populations of larvae may be artificially infected with E. maimaiga. Three of these methods involve infecting larvae in the laboratory and then releasing them into the environment. Larvae may be infected by dipping them into conidial suspensions, by exposing them to sporulating cadavers discharging conidia, or by injecting them with protoplasts directly into the hemocoel. When infected larvae are released, healthy larvae are presumably infected by conidia produced by cadavers of the released individuals (Hajek and Roberts, 1991). Success or failure using these methods is at least partly dependent on the number of larvae introduced into field populations. A release of 500 fungal-infected caterpillars in 1990 led to a mean fungal infection level of 20.2% in one experimental plot (Hajek and Roberts, 1991). While these methods are relatively effective, they are labour-intensive and may not be feasible for infecting field populations on a large-scale basis.

Another method of infecting field populations of gypsy moth is by releasing resting spores into the environment. These spores have an obligate dormant period and, upon germination, produce infective germ conidia. Ferron (1978) suggested that, although entomophthoralean resting spores are an excellent choice for use in pest control, discouraging results in past research and a lack of methodology to induce rapid and simultaneous germination of resting spores have prevented further exploration into their potential use. Hajek and Roberts (1991) reported successful transmission of the pathogen to field populations through the distribution of soil containing resting spores in field plots. Such treatments yielded fungal mortality levels of about 34%. When over-wintering resting spores were introduced into an experimental plot and regularly

watered, over 90% of the larvae died (Hajek and Roberts, 1991). Introduction of resting spores into the environment is labour-intensive, especially when plots have to be irrigated. However, interest exists in automating this method of introduction. Development of an aerial application method of introducing dehydrated resting spores into the environment may be a future research option in the field of E. maimaiga research (Tyrrell, pers. comm).

This thesis is intended to provide information about the status of gypsy moths in the Niagara Region in 1991 and to evaluate a method of introducing E. maimaiga-infected larvae into a local population of gypsy moth. Investigations about relationships between moth density and percent fungal infection and between occurrence of fungus and occurrence and intensity of virus were performed. The methods by which these objectives were accomplished and the results that were obtained are presented in the next two sections.

Methods

In this research a combination of laboratory and field studies was performed in order to study aspects of the population ecology of host-pathogen interactions. Host and pathogen used in the research paradigm were the gypsy moth and a specific entomopathogenic fungus, E. maimaiga. Gypsy moth populations in different areas of the Niagara Region were censused between 1991 and 1993. In 1992, an entomopathogenic fungus, Entomophaga maimaiga, was introduced into one of these sites, Willoughby Marsh, by releasing gypsy moth larvae which had been injected with fungal protoplasts in the laboratory at Brock University. Later collections and examination of field larvae over 1992 and 1993 compared the extent of mortality in the field due to the fungus between release and control sites. Lastly, data were collected with regard to the occurrence of E. maimaiga and the occurrence and intensity of nuclear polyhedrosis virus (NPV) infection.

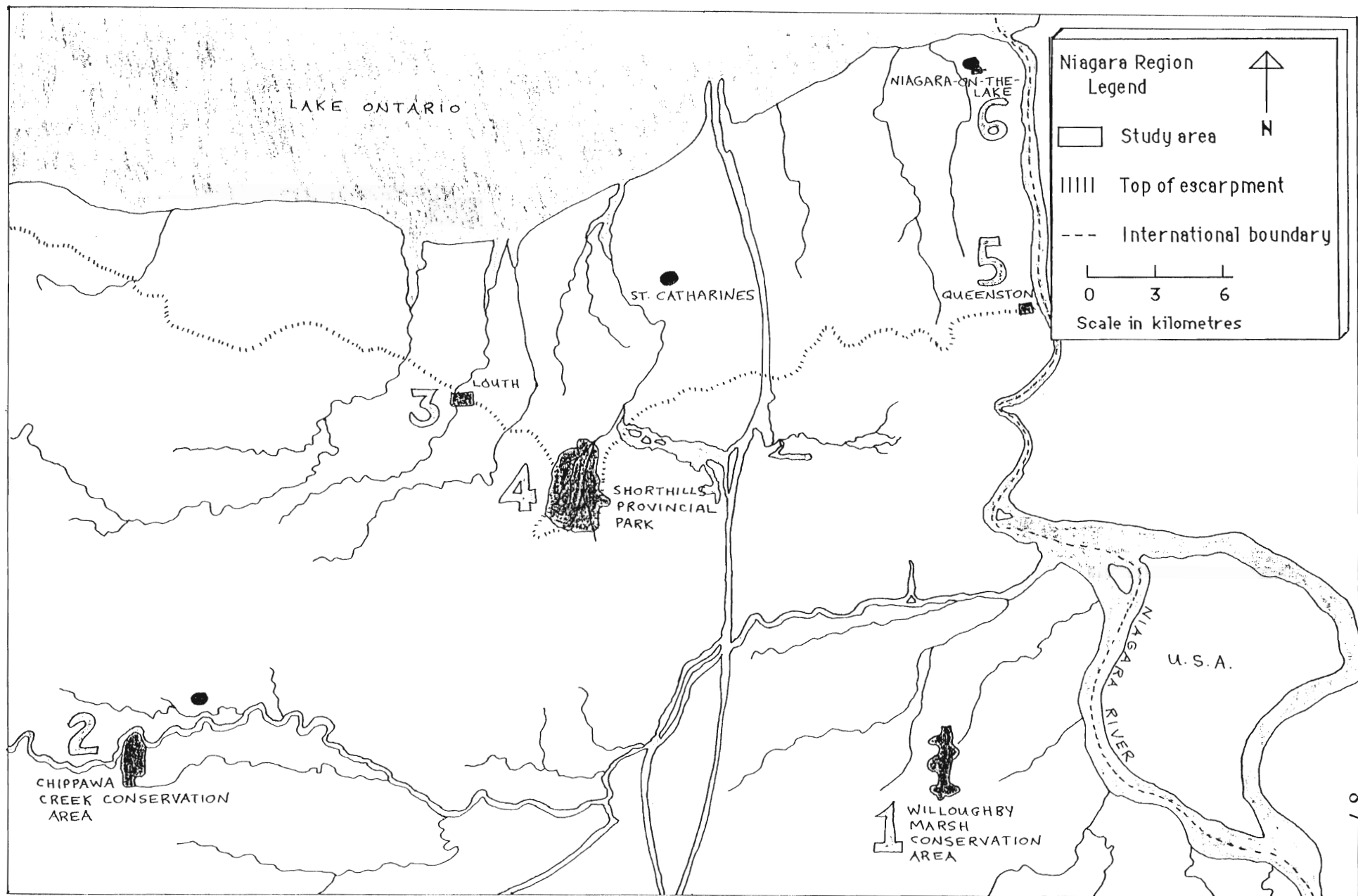
Gypsy Moth Populations in the Niagara Region

Study Areas

Six areas in the Niagara Region were chosen to study population dynamics of the gypsy moth. All supported populations of gypsy moth, were easily accessible by car from Brock University and contained stands of oak (Quercus spp.), poplar (Populus spp.) and/or birch (Salix spp.) which are favoured food species of gypsy moth larvae (Martinat et al. 1987). The six areas were Louth Conservation Area (32 hectare [ha]), a woodlot on the Niagara Escarpment located near Queenston Heights (32ha), another woodlot located in the town of Niagara-on-the-Lake (32ha), Shorthills Provincial Park (688ha), Chippawa Creek Conservation Area (148ha), and Willoughby Marsh Conservation Area (231ha) (Figure 10). All are public areas, used primarily for

Figure 10. Map of the Niagara Region showing locations of the six study sites:

1. Willoughby Marsh Conservation Area;
2. Chippawa Creek Conservation Area;
3. Louth Conservation Area;
4. Shorthills Provincial Park;
5. Queenston; and
6. Niagara-on-the-Lake.



recreational activities, except for Chippawa Creek Conservation Area which is used both for recreation and for logging. Permission to work in these areas was obtained from the Ontario Ministry of Natural Resources (OMNR), the Niagara Regional Conservation Authority (NPCA) and the Niagara Parks Commission (NPC). These organizations also provided information about the size and location of gypsy moth infestations in each area, and details regarding past and present spraying programs.

Estimating Gypsy Moth Population Density

Locations of sampling plots were determined on maps (scale=1:10,000) of each of the six areas. Parallel transect lines, 200m apart, were drawn to scale on each map generally in an east-west direction. Sample plots, marked 100m apart on each transect line, yielded a total of 16 plots each for Louth and Queenston, 10 for Niagara-on-the-Lake, 78 for Shorthills, 20 for Chippawa Creek (Figure 11) and 73 for Willoughby Marsh (Figure 12).

Ground plots (0.01ha), called modified Kaladar plots (MKPs) (Nealis and Erb, 1993) were established at each sampling plot. MKPs were aligned along the line of travel usually in an east-west direction. They were constructed by marking the centre and each corner of a 10m x 10m plot with wooden stakes. Each stake was marked with fluorescent paint for ease of identification.

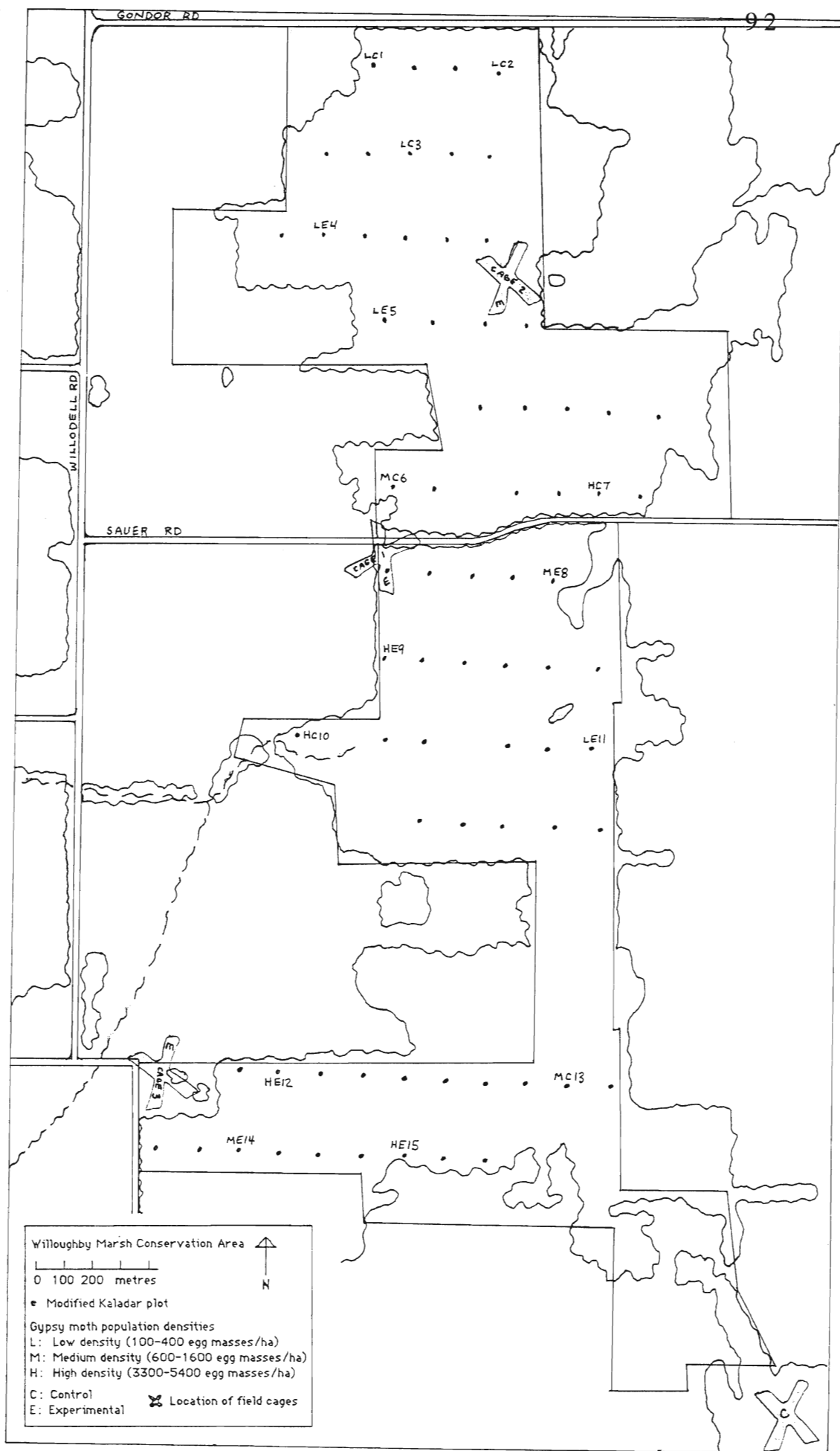
Since counts are more accurate after trees have lost their leaves in the fall (Liebhold *et al.*, 1993), egg masses were counted in each plot between late November and early April on days when no snow lay on the ground. Only egg masses which had been deposited the summer before were counted. When first deposited, egg masses are buff-coloured and velutinous. They may remain intact attached to

Figure 11. Map of Chippawa Creek Conservation Area (Wellandport: concession 7, part lots 46 and 47) showing locations of study plots.



Figure 12.

Map of Willoughby Marsh Conservation Area (Niagara Falls: part concessions 5 and 6, part lots 1 to 6) showing locations of study plots, three experimental cages and one control cage.

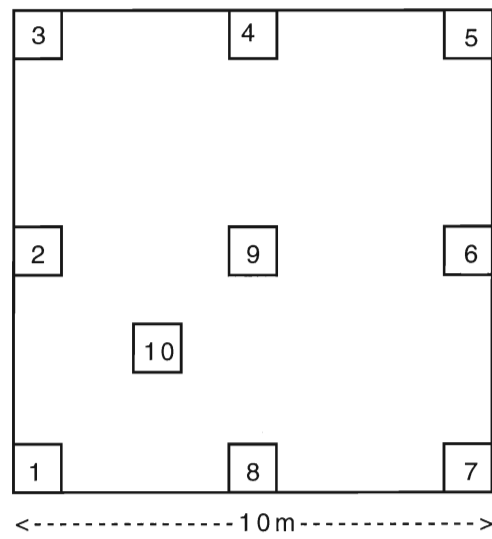


structures for several years but, as they age, their appearance becomes increasingly bleached and ragged-looking. Plots may contain egg masses which have accumulated over several years. However, differences in colour and texture permit one to distinguish between new and old egg masses.

Within each MKP, egg masses were counted both above ground level and at ground level. For above ground level counts, all trees, saplings, bushes and other structures within the plot were examined carefully for egg masses. Egg masses on branches extending beyond the plot were included if the main trunk lay within the prescribed area. Trees on the plot perimeter were included in the data collection if the centre of the tree bole was within the sampling plot. Binoculars were used as needed for viewing egg masses on taller trees. Density of egg masses at ground level was estimated by sampling 10 1m² sub-plots within the main MKP (Figure 13). Ground counts were multiplied by 10 and then combined with their respective above ground level counts. The subsequent total when multiplied by 100 was an estimate of gypsy moth egg masses per hectare (Appendix 1).

All six study sites were censused twice in 1991. Preliminary explorations were made in the spring of the year to assess each site for occurrence and abundance of gypsy moth. Accurate egg mass counts were made in the fall of the year. Because egg masses were scarce in 1991 in four of the six sites, Louth, Queenston, Niagara-on-the-Lake and Shorthills, counts were not conducted in these sites in 1992 or 1993. Chippawa Creek and Willoughby Marsh Conservation Areas were selected for more intensive study in these two years. Willoughby Marsh was selected as the principal experimental area because of greater variation in egg mass counts, its larger area, and

Figure 13. Locations of ten mini-plots in a modified Kaladar plot.



absence of logging disturbances. The number of plots censused in Willoughby Marsh in 1992 was reduced from 73 to 45 plots (Figure 12) corresponding to 3x the number of primary fungus release plots (see later section). Egg mass counts in all plots were conducted in 1993. A reduced number of plots was also censused in Chippawa Creek in 1992 and all plots were censused in 1993 (Figure 11) in order to compare annual changes in population estimates to estimates obtained in Willoughby Marsh. Population estimates (egg masses per ha) were therefore recorded for three successive years in the two sites.

Fungus-Moth Interactions

Experimental Release of the Fungus, E. maimaiga

United States Agricultural Research Service Collection of Entomopathogenic Fungi (ARSEF) no. 2779, from Massachusetts, a virulent isolate of E. maimaiga, (Walsh, pers. comm.), was obtained for use in this study from the laboratory of Dr. J. Silver at the Scarborough campus of the University of Toronto. Protoplasts of ARSEF 2779 were maintained in the dark at 4°C in a sterile liquid nutrient medium composed of Grace's Insect Cell Culture Medium (GICCM) and 5% fetal bovine serum (FBS) (Gibco Laboratories 350-1590AJ and 200-6140PE, respectively). Late instar, pathogen-free gypsy moth larvae were obtained from the rearing facility of the Forest Pest Management Institute (FPMI) in Sault Ste. Marie. The larvae were maintained in the laboratory on a 14.5L:9.5D photoperiod at approximately 23°C and were fed high wheat germ gypsy moth diet (Bio Serv product no. 9628). Ten-20 individuals were housed in 500ml opaque plastic containers with perforated lids.

Establishing Suitability of Test Isolate for Field Study

The isolate of ARSEF 2779 obtained for use in the study had been previously sub-cultured seven or eight times in Dr. Silver's laboratory. Since cultures of E. maimaiga suffer a decline in virulence with repeated sub-culturing (Hajek *et. al.*, 1990b), it was necessary to test the pathogenicity and the virulence of the test isolate prior to the test release.

Protoplast cultures may be maintained in viable condition by periodic transfer of small amounts of the original culture to fresh medium (Tyrrell and Macleod, 1972). The original cultures from Scarborough were subcultured in order to establish a healthy population of protoplasts. New culture media (9.5ml GICCM and 0.5ml FBS) was prepared in 50ml tissue Nunclon® culture flasks using aseptic technique. After approximately 24h at room temperature, flasks with no signs of bacterial contamination were selected for further use. Some original protoplast culture medium (0.02ml) was pipetted into each sterile culture flask, again under aseptic conditions. Caps of flasks were screwed on loosely to maintain aerobic conditions. Newly prepared flasks were kept in the dark at 22-25°C for 48-60h in order to ensure rapid population growth of protoplasts. Darkness facilitates rapid protoplast growth (Walsh, pers. comm.). On Day 4, protoplast density (protoplasts per μ l) was estimated using a hemocytometer.

Four estimates of protoplast density in the new sub-cultures were determined and a mean density estimate was obtained. The number of protoplasts likely to cause infection was decided in accordance with studies performed by Hajek *et al.* (1990a,b) who successfully infected larvae with approximately 500 protoplasts of E. maimaiga. Consequently, approximately 8.4 μ l of medium, containing

400-600 protoplasts, were introduced into the hemocoel of 39 late instar larvae by injecting each one at the base of a proleg using a 10cc syringe and a 23ga needle installed in a syringe microburet (Micro-Metric Instrument Co., Tampa, Fla., Model #SB-2). The larvae were then kept without food for 24h to reduce the risk of septicemia caused by accidental puncture of the gut wall by the needle and to prevent larvae from ingesting fungicides present in their diet. Once this deprivation period ended, larvae were placed back in their home containers and monitored daily.

Dead caterpillars were removed and placed in individual petri dishes on moistened filter paper. Cadavers were observed daily and signs of sporulation were recorded. At least seven days after death, portions of cadavers were macerated and stained with lactophenol-cotton blue (BDH Inc. RO3465) before being microscopically examined (x100) for the presence of conidia or resting spores of E. maimaiga. The number of cadavers positive for E. maimaiga and the mean time to death (in days) were recorded.

Field Release of Fungal-Infected Larvae

In the spring of 1992, gypsy moth eggs, produced by laboratory-reared mated females at the FPMI, were hatched out in the laboratory. They were raised to the third, fourth and fifth instar stage on artificial diet at 23°C on a 14.5L:9.5D photoperiod until preparations for their injection and release were complete.

A number of plots (n=15) in Willoughby Marsh were chosen as release points on the basis of the previous year's egg mass counts. All plots were at least 225m apart as late instar larvae are reportedly capable of traveling up to 250m (Hajek, pers. comm.). Release points were, therefore, separated as much as possible to minimize cross-

contamination of plots. The plots were designated either experimental (n=8) or control (n=7) and represented various density levels (Table 1).

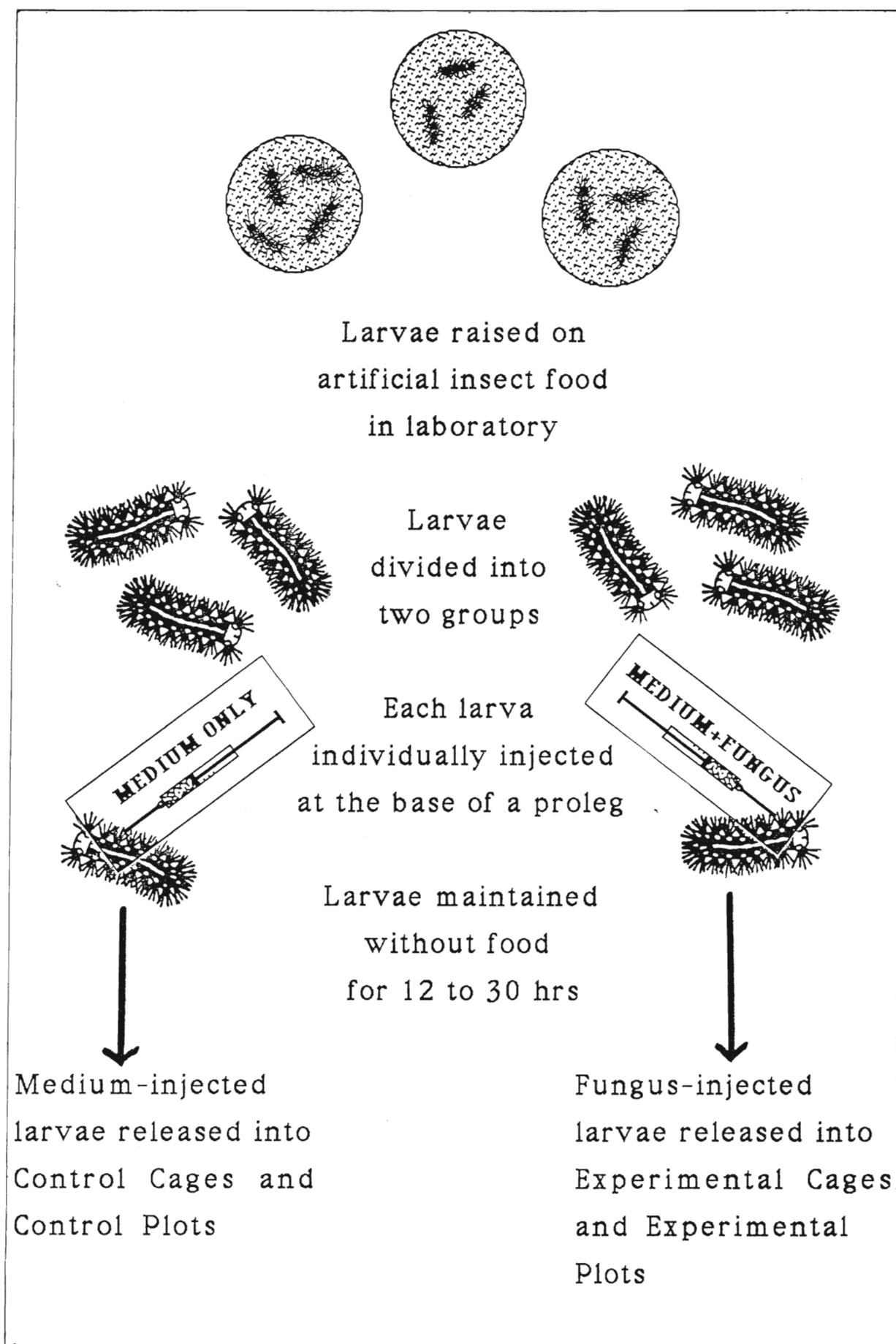
Approximately 2200 healthy larvae (third to sixth instars) were divided into 15 groups. Experimental larvae were injected with 300-600 protoplasts in 8 to 17 μ l of medium. Control larvae were injected with equivalent volumes of medium only. Injection procedures are summarized in Figure 14. After being injected, all larvae were maintained without food for a period of 12-30h. A certain percentage of larvae (6.8% for the control groups and 16.8% for the experimental) died during this period, perhaps as a result of trauma caused by the injection. These fatalities were removed, which left for release 129 larvae per experimental plot and 125 per control plot. Approximately 12-30h after injection, over a three day period in late June of 1992, these laboratory-injected larvae were released into Willoughby Marsh by placing them on tree trunks, preferably of oak, in each of the designated plots. After death, these laboratory larvae would serve as a means of infecting field population with the entomopathogenic fungus.

Fungal-injected larvae may take a mean of 8.5d to die in the laboratory (Hajek et al., 1990b). Time to death might differ under field conditions because of daily fluctuations in such parameters as temperature and humidity. Therefore, to monitor time to death under field conditions, small groups of fungal-injected larvae (n=19-28) were placed into three screened cages (0.39m x 0.39m x 0.39m) in various parts of Willoughby Marsh (Figure 12). To see how uninfected laboratory larvae, which had been subjected to an injection regime, fared in the natural environment, control larvae (n=88), which had been

Table 1. Density classifications of 15 MKPs chosen as study plots in Willoughby Marsh Conservation Area.

Density class	Egg mass count (egg masses/ha)	No. of plots	
		Experimental	Control
low	100-400	3	3
medium	600-1600	2	2
high	3300-5400	3	2

Figure 14. Diagrammatic summary of injection methodology.



injected with medium only, were placed into a screened control cage (0.91m x 0.61m x 0.51m). Small branches of oak were inserted through a single sealed opening in each cage to provide food for the caged larvae. The three experimental cages were checked every two to four days for two weeks. The control cage was monitored every two to four days until all larvae died, 39d after initiation. Rate of mortality in each cage was assessed.

Once the caged experimental larvae had died, it was assumed that all released experimental larvae had died as well and field collections of live larvae in each of 15 plots in Willoughby Marsh were begun. The probability of mistaking laboratory-reared for field individuals and thus overestimating mortality caused by the fungus was thus minimized

Collections of Field Larvae

Late instar larvae climb down from tree tops and aggregate in cracks and crevices during the day, especially at low densities. These hiding places may be simulated by tying burlap sacks around tree trunks (Liebhold *et al.*, 1986). Using this method, it is possible to capture larvae, even at low densities (Weseloh, 1987). To capture larvae in this study, burlap flaps were placed 1-1.5m from the ground around two or three mature tree trunks, preferably oak, within each of the 15 plots as depicted in Figure 15.

Collections of field larvae began in mid-July, 14 to 16d after the initial release, once the majority of caged larvae were dead. Two larvae, one from each of two experimental cages, survived and exhibited no subsequent signs of fungal infection. Sample plots were visited two to three times over the next two weeks and the burlap sacks were examined for larvae. Groups of 10-30 live larvae were

Figure 15. Burlap sack placed around a mature oak tree for collection of gypsy moth larvae.



collected on each sampling occasion. Larvae found on nearby trees and bushes within the plot were also collected. By late July, most field larvae had pupated and no more remained to be collected.

Field-collected larvae were placed in groups of 10-30 in 500ml opaque plastic containers, fed artificial diet and monitored daily for death or pupation. Those individuals that did not emerge from a pupa after approximately one month were presumed dead. Dead larvae and pupae were placed on water-saturated filter paper in individual petri dishes and observed daily for the occurrence of sporulation. This may occur within 14h after death of the host (Hajek et al., 1990c). At least one week after death, microscopic slides of macerated portions of each cadaver were stained with lactophenol-cotton blue and the body contents examined for the presence of conidia or resting spores of E. maimaiga as described earlier.

Willoughby Marsh plots were examined the year following the release of the fungus (see following) to determine whether the released pathogen had established itself in its host population. In late June and July of 1993, control and experimental sites were sampled about four times in 2.5wk beginning eight weeks after larval hatch. Groups of 10-30 larvae were collected each time from each of 15 plots and returned to the laboratory where they were maintained on an artificial diet. Dead individuals were isolated and examined for evidence of the fungus as was described earlier.

For comparative purposes, larvae were also collected from Chippawa Creek, about 32km west of Willoughby Marsh. Time constraints and reduced larval populations limited collections to two sampling sites at opposite ends of Chippawa Creek (Figure 11). A total of 139 larvae were collected from under burlap sacks in these two

plots on three different occasions between late June and mid-July, at which time most individuals had pupated. Collected field larvae were treated the same as larvae from Willoughby Marsh. To prevent cross-contamination of larvae from the two sites, Willoughby Marsh and Chippawa Creek were visited on different days and collections of larvae were housed in separate rooms in the laboratory. Records were kept of time to death and emergence of adults for all collected larvae.

Other Sources of Gypsy Moth Mortality

Other sources of mortality influence populations of gypsy moth. Of these, the gypsy moth nuclear polyhedrosis virus (NPV) and various parasitoids were also noted in this study.

One of the most successful introduced pathogens, the nuclear polyhedrosis virus (NPV), is commonly found throughout gypsy moth populations (Hajek and Roberts, 1992). Although the relationship between gypsy moth virus and fungus is not well understood, it is known that they may be viable at the same time within one individual. Death occurs more quickly in a larva infected with one organism if it becomes infected with the other (Hajek, pers. comm.) Studies in the past have noted the presence or absence of PIBs (Weseloh and Andreadis, 1992 a,b) and attributed cause of death to NPV if any PIBs were observed upon microscopic examination of a cadaver (Murray et al., 1991). Campbell and Podgwaite (1971) considered polyhedral virus inclusion bodies to be pathogenic if they were revealed at x1000 magnification in numbers greater than or equal to ten per field in a stained larval smear. The choice of "ten" was made as an arbitrary number for separating insects which probably contained enough polyhedra for the virus alone to have caused death from those in which the low number of polyhedra did not allow a definite diagnosis.

In this study, the degree of NPV infection in a sub-sample of specimens was assessed in order to determine if a relationship exists between number of viral particles and occurrence of fungus. Gypsy moth cadavers that were positive for E. maimaiga were crushed and smeared onto microscope slides. The slides were allowed to dry for at least one hour before being placed into a bath of buffalo black stain (naphthol blue black from Allied Chemical 484) for 10min at 40-45°C. The slides were examined (oil immersion x1000) for the presence of polyhedral inclusion bodies (PIBs) which are indicative of the presence of NPV (Cunningham, pers. comm.; Hajek and Roberts, 1992). The number of PIBs in ten randomly selected fields was counted for each specimen. In the same manner, a random subsample of those gypsy moth cadavers that were negative for E. maimaiga was also examined for the presence of PIBs.

Some dipteran and hymenopteran parasitoids were noted in field-collected larvae. Records were kept of their incidence, and emerging dipteran and hymenopteran adults were preserved in 70% alcohol for later identification. Representative individuals of each type were sent to J. Poirier at Agriculture Canada in Ottawa for identification.

Statistical Analysis of Data

All data sets relating to gypsy moth density and fungal mortality were tested for normality using the Kolmogorov-Smirnoff (Lillefors) test (Wilkinson, 1989). None of the frequency distributions proved normal even after the application of various transformations. Therefore, the non-parametric statistical tests i.e. the Mann-Whitney U-test and the Kruskal-Wallis test were used in subsequent analyses. When calculations using the Kruskal-Wallis test indicated that the null hypothesis should be rejected, non-parametric Tukey-type

multiple comparison tests were used to detect statistical differences between sets of data. Relationships between host density and fungal infection and between fungal infection in the 2 years, 1992 and 1993, were examined using Spearman rank correlations. Three dimensional contingency tables were used to analyze the prevalence (% occurrence) of gypsy moth NPV. The three levels were: presence or absence of fungus; presence or absence of virus; and occurrence in experimental or control plots. Non-parametric two-way ANOVAs, using ranked data, were used to examine the effect of percent fungal infection and plot type on the intensity of gypsy moth NPV infection.

Results

Gypsy Moth Population Density Estimates in the Niagara Region

Population density estimates in the six study sites in 1991 varied considerably. Significant differences in density in the six sites were detected using the non-parametric Kruskal-Wallis test ($H=29.81$; $df=5$; $p=0.0001$). Figure 16 illustrates frequency distribution histograms for four of the sites. Low population densities (egg masses/ha) were estimated for Louth Conservation Area (6.3 ± 2.5 [Mean \pm SD]), Queenston (12.5 ± 34.2) and Niagara-on-the-Lake (20 ± 63.2). A non-parametric Tukey-type multiple comparison test indicated that there was no significant difference between densities in these three low density sites ($Q < 2.1$; $k=6$; $p > 0.5$). Variation within all three sites was similar with numbers of egg masses ranging from 0-2 per sampling plot. The three larger sites, Shorthills Provincial Park, Chippawa Creek Conservation Area and Willoughby Marsh Conservation Area showed greater variance within sites and higher population densities overall. Frequency distributions were skewed with most plots within sites having few or no egg masses and few plots having over 3200 egg masses/ha (Figures 16-18). Mean population densities of egg masses were 175 ± 432.1 , 610 ± 851.6 and 665.8 ± 1356.8 egg masses/ha for Shorthills, Chippawa Creek and Willoughby Marsh respectively. The Tukey test indicated that, while density in Chippawa Creek and in Willoughby Marsh were comparable ($Q < 2.1$; $k=6$; $p > 0.5$), density in Chippawa Creek was significantly higher than in Shorthills ($Q=3.0$; $k=6$; $p=0.038$).

A reduced number of plots was censused both in Chippawa Creek and Willoughby Marsh in 1992 in conjunction with the experimental release of E. maimaiga. Post-release estimates of population density

Figure 16. Frequency distributions of gypsy moth egg mass density in (a) Louth Conservation Area, (b) Queenston Heights, (c) Niagara-on-the-Lake and (d) Shorthills Provincial Park in 1991 (n=number of plots).

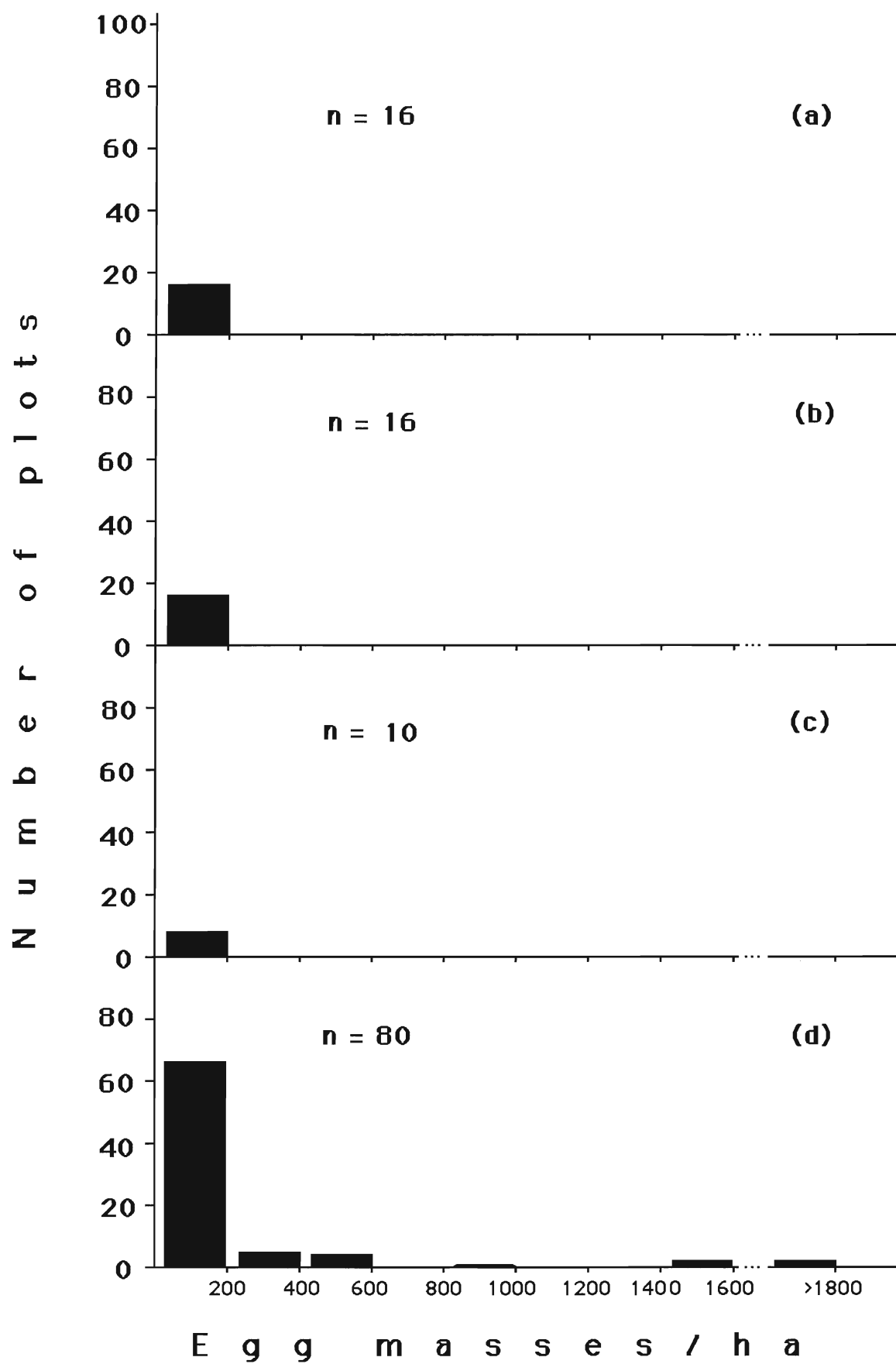


Figure 17. Frequency distributions of gypsy moth egg mass density in Chippawa Creek Conservation Area in 1991, 1992 and 1993 (n=number of plots).

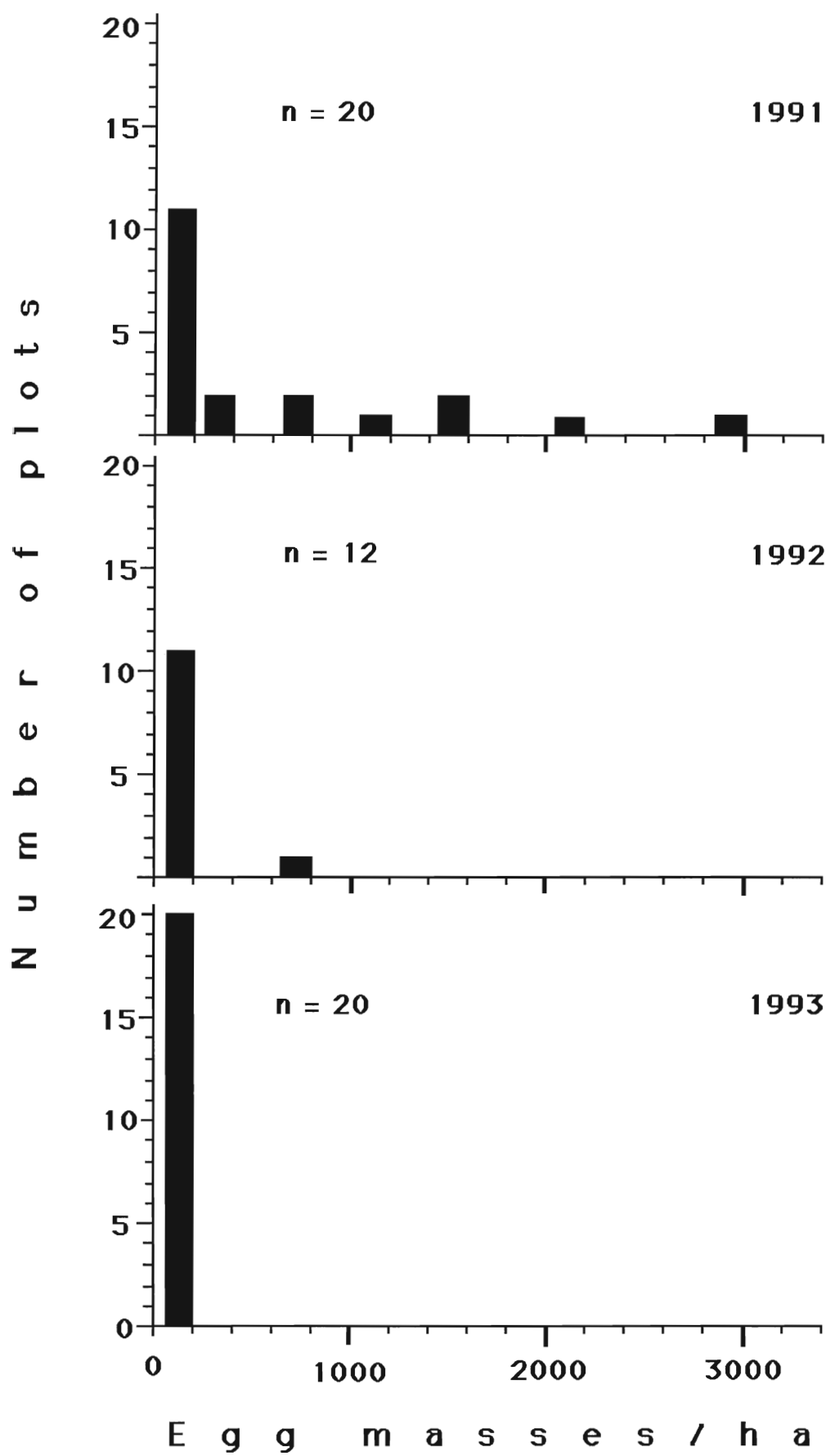
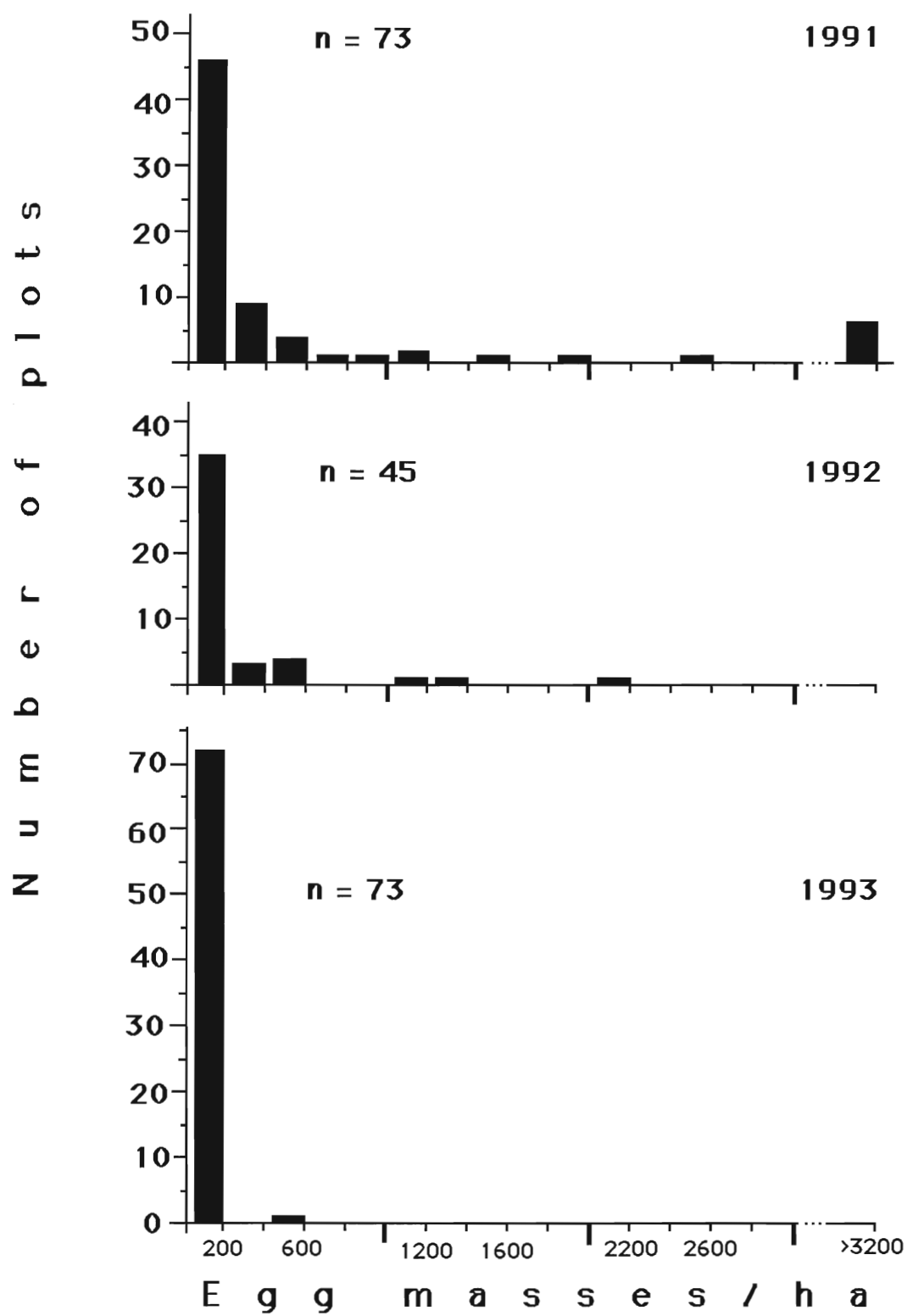


Figure 18. Frequency distributions of gypsy moth egg mass density in Willoughby Marsh Conservation Area in 1991, 1992 and 1993 (n=number of plots).



were calculated for all plots in both sites in 1993. These data are reported in a later section.

Establishing Suitability of Test Isolate for Field Study

Before the fungus was released into the field, the virulence of test isolate 2779 was evaluated in the laboratory. The survivorship curve of 39 gypsy moth larvae infected in the laboratory with protoplasts of this isolate is shown in Figure 19. Ninety-two percent (n=36) of the larvae died within 15d of being injected. Three larvae survived and eclosed to adulthood. The mean time to death was 8.8 ± 2.8 d. Most larvae (n=11) died on the seventh day after injection. Microscopic examination in search of resting spores and/or conidia revealed evidence of E. maimaiga in 64.1% of cadavers.

Field Release of Fungal-Infected Larvae

Data on the survival of non-infected larvae injected with culture medium only and placed into a control cage in the field are shown in Figure 20. Over 52% of these larvae were dead within two days of being placed in the field. Most larvae were dead by the sixth day post-release. The mean time to death was 8.6 ± 9.9 d. All larvae were dead by the 39thd post-release.

Survivorship curves for fungus-infected larvae in each of three experimental cages, cages 1-3 are illustrated in Figure 21. Percent mortality per cage varied from 96.4 to 100%. One larva in each of two of the cages survived the observation period and showed no evidence of E. maimaiga when later examined in the laboratory. Most larvae in cages 1 and 2 died on the fourth day post-release and between the second and fourth day in cage 3. Mean times to death were 6.8 ± 3.2 , 3.3 ± 0.9 and 5.7 ± 4.6 d for cages 1-3 respectively.

Figure 19. Survivorship curve of larvae injected in the laboratory with E. maimaiga isolate ARSEF 2779.

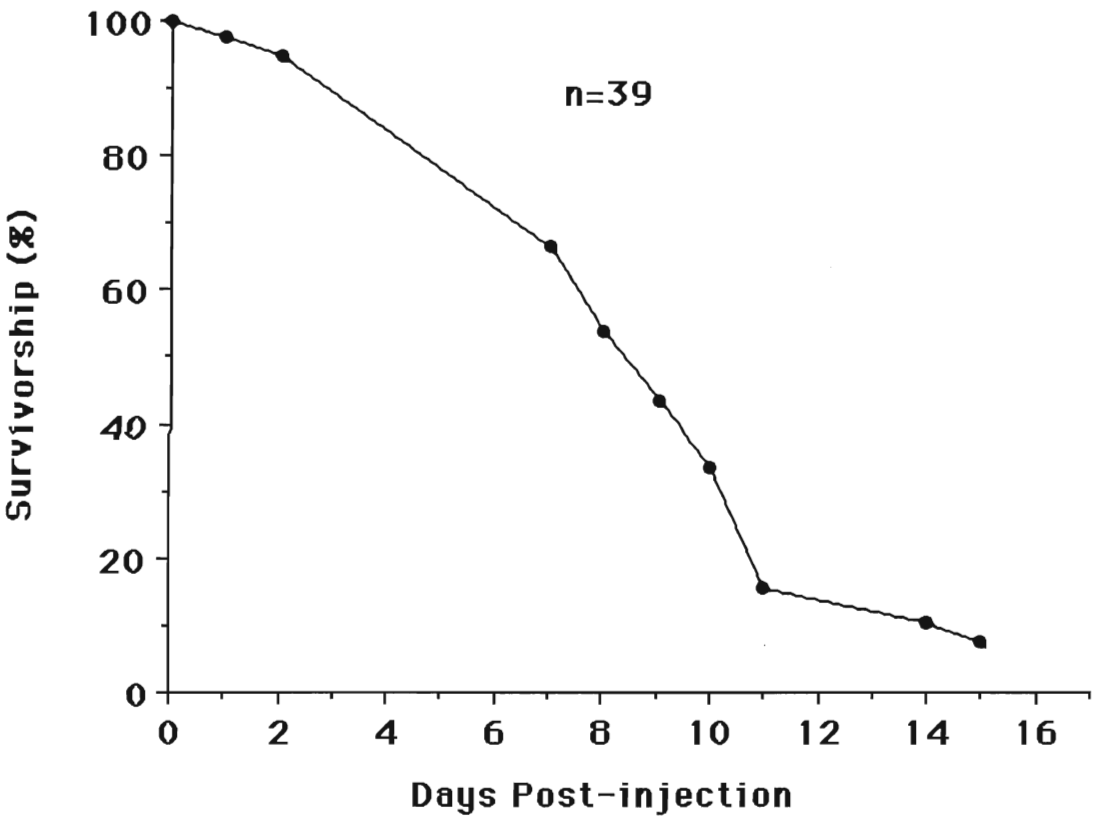


Figure 20. Survivorship curve of medium-injected larvae maintained in control cage in Willoughby Marsh Conservation Area in 1992.

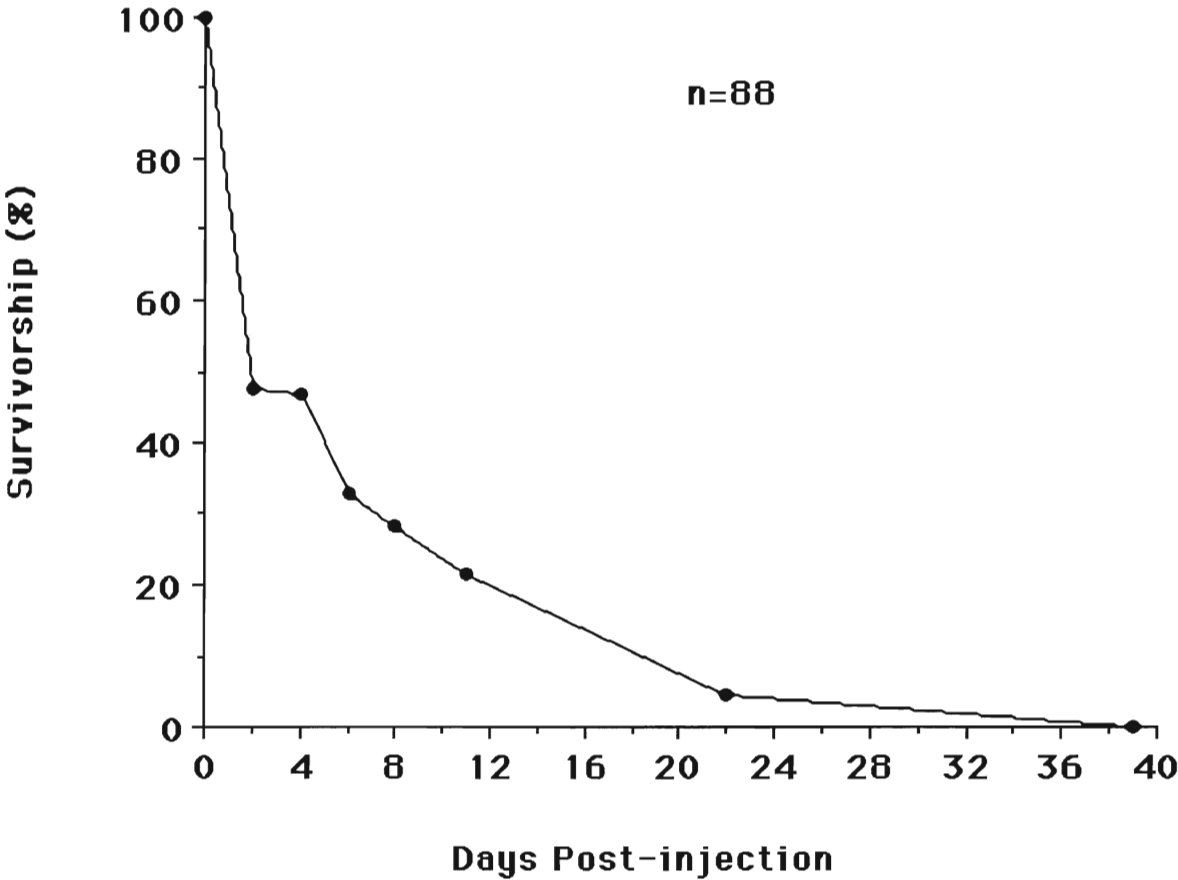
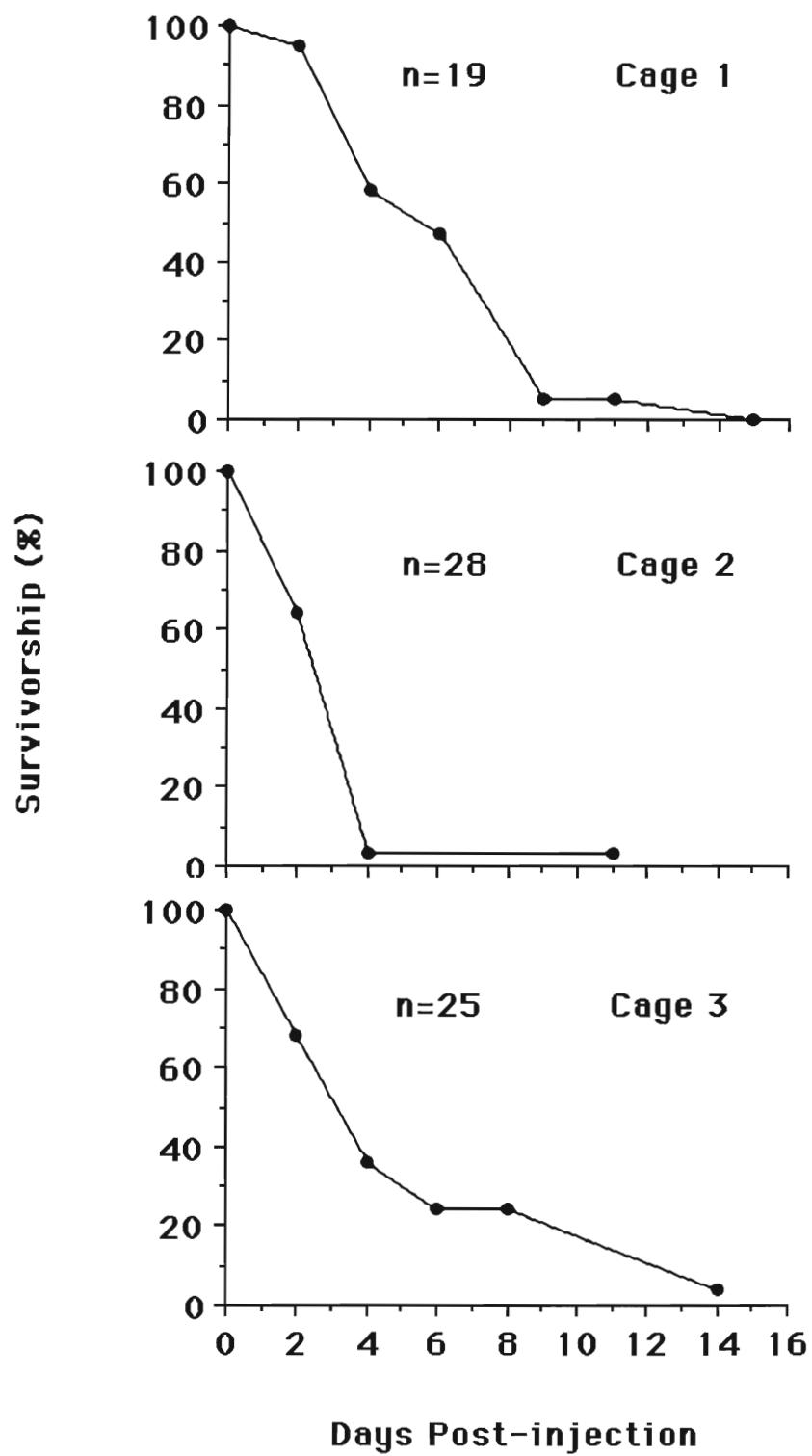


Figure 21. Survivorship curves of fungal-infected larvae maintained in three cages in Willoughby Marsh Conservation Area in 1992.



There were significant differences ($H=29.80$; $df=4$; $p=0.0001$) in mean times to death among larvae housed in different locations i.e. the laboratory (fungus-injected), the control cage (medium-injected) and field cages 1-3 (fungus-injected). When compared to mean time to death in the laboratory, mean time to death in cage 1 was not significantly different ($Q<2.0$; $k=5$; $p>0.5$) but mean time in the control cage was ($Q>3.9$; $k=5$; $p<0.001$). Larvae died more slowly in the laboratory than in field cages 2 and 3 ($Q>3.9$; $k=5$; $p<0.001$; $Q=3.5$; $k=5$; $p=0.005$ respectively). Larvae in cages 2 and 3 died in about the same time ($Q=2.0$; $k=5$; $p=0.5$). Larvae lived longer in cage 1 than in cage 2 ($Q=3.2$; $k=5$; $p=0.014$). There were no significant differences in mean times to death between larvae in the control cage and in cages 1-3 ($Q=2.1$; $k=5$; $p=0.38$; $Q=2.4$; $k=5$; $p=0.17$; $Q<2.0$; $k=5$; $p>0.5$ respectively). These results are confusing but clarification will be provided in the discussion section (see section entitled Survival of Non-Infected and Infected Larvae in Cages).

Collections of Larvae in Willoughby Marsh

No external evidence of E. maimaiga was observed on gypsy moth cadavers in Willoughby Marsh in 1991. After release of the fungus in 1992, the presence of E. maimaiga was confirmed microscopically in $13.7\pm13.5\%$ of the larvae ($n=533$) collected in Willoughby Marsh. Table 2 illustrates the frequency of occurrence of E. maimaiga in larvae collected from the seven control plots. Two of the plots showed no evidence of this fungus. The overall mean proportion of larvae positive for E. maimaiga in control plots was $5.1\pm4.8\%$. Table 3 illustrates the frequency of occurrence of E. maimaiga in larvae collected from the eight experimental plots where fungus had been released one month earlier. One plot showed no evidence of E.

Table 2. Occurrence of E. maimaiga in gypsy moth larvae and pupae collected from control plots in Willoughby Marsh Conservation Area in 1992, the year the fungus was released.

Plot No.	Number Infected		Total Number Collected	% Infected with <u>E. maimaiga</u>
	Larvae	Pupae		
C1	1	3	52	7.69
C2	4	0	46	8.70
C3	0	0	41	0.00
C6	3	2	39	12.82
C7	2	0	48	4.17
C10	0	0	16	0.00
C13	1	0	38	2.63
Total	11	5	280	

Table 3. Occurrence of E. maimaiga in gypsy moth larvae and pupae collected from experimental plots in Willoughby Marsh Conservation Area in 1992, the year the fungus was released.

Plot No.	Number Infected		Total Number Collected	% Infected with <u>E. maimaiga</u>
	Larvae	Pupae		
E4	5	0	40	12.50
E5	5	0	43	11.63
E8	10	0	21	47.62
E9	4	0	18	22.22
E11	2	1	17	17.65
E12	0	0	36	0.00
E14	6	0	40	15.00
E15	9	0	38	23.68
Total	41	1	253	

maimaiga, while in another, almost half of all larvae collected exhibited fungal infection. A mean of $10.8 \pm 13.8\%$ of all larvae collected in experimental plots were positive for E. maimaiga. The proportion of larvae that were infected with fungus was significantly higher in experimental than in control plots (Mann-Whitney U-test: $Z = -2.32$; $p = 0.02$).

In 1993, a total of 486 larvae were collected from the same 15 plots in Willoughby Marsh. The presence of E. maimaiga was confirmed in $46.8 \pm 25.2\%$ of these larvae. In control plots, $47.9 \pm 18\%$ of them were infected (Table 4) while in experimental plots, $45.4 \pm 31.6\%$ were infected (Table 5). The proportion of infected larvae was not significantly different between experimental and control plots ($Z = -0.12$; $p = 0.91$).

Significantly more larvae from Willoughby Marsh were infected with E. maimaiga in 1993 than in 1992 ($Z = -3.7$; $p = 0.0002$). There was a significant increase in the percentage of infected larvae from control plots between 1992 and 1993 ($Z = -3.13$; $p = 0.0017$). The difference between those infected from experimental plots between 1992 and 1993 showed borderline significance ($Z = -1.94$; $p = 0.052$).

Collections of Larvae in Chippawa Creek

In 1993, a total of 139 larvae from two plots in Chippawa Creek Conservation Area were examined for the presence of E. maimaiga. Infection levels in the two plots were 4.6% ($n = 65$) and 16.2% ($n = 74$), giving a mean of $10.4 \pm 8.2\%$. Percent infection which occurred in Chippawa Creek in 1993 was close to being significantly lower than that which occurred in Willoughby Marsh during the same year ($Z = -1.9$; $p = 0.053$).

Table 4. Occurrence of E. maimaiga in gypsy moth larvae and pupae collected from control plots in Willoughby Marsh Conservation Area in 1993, the year after the fungus was released.

Plot No.	Number Infected		Total Number Collected	% Infected with <u>E. maimaiga</u>
	Larvae	Pupae		
C1	42	0	83	50.60
C2	14	0	31	45.16
C3	23	1	29	82.76
C6	33	0	74	44.59
C7	3	0	11	27.27
C10	8	0	25	32
C13	19	9	53	52.83
Total	142	10	306	

Table 5. Occurrence of E. maimaiga in gypsy moth larvae and pupae collected from experimental plots in Willoughby Marsh Conservation Area in 1993, the year after the fungus was released.

Plot No.	Number Infected		Total Number Collected	% Infected with <u>E. maimaiga</u>
	Larvae	Pupae		
E4	5	0	10	50.00
E5	39	1	66	60.61
E8	37	0	42	88.10
E9	2	1	10	30.00
E11	1	0	6	16.67
E12	10	0	31	32.26
E14	6	0	7	85.71
E15	0	0	8	0.00
Total	100	2	180	

Density vs. Larval Infection (%) in 15 Plots in Willoughby Marsh

Figure 22 shows a scatter diagram of the percentage of larvae infected with the fungus plotted against density of each plot for 1992. There was no correlation between the proportion of larvae infected with the fungus and host population density for either control ($r_s = -0.24$; $p = 0.56$) or experimental plots ($r_s = 0.05$; $p = 0.9$).

Figure 23 shows a scatter diagram of the percentage of larvae infected with fungus plotted against density of each release plot for 1993, the year after the release of E. maimaiga. There was no correlation between percent infection and host density for either control ($r_s = -0.52$; $p = 0.20$) or experimental plots ($r_s = 0.69$; $p = 0.069$). When data from low density plots were omitted from the analysis and a comparison was made only between medium and high density plots, a significantly higher proportion of larvae from the medium density plots were positive for E. maimaiga ($Q = 3.0$; $k = 3$; $p = 0.016$).

Figure 24 shows a scatter diagram of the percentage of larvae infected with fungus in 1992 plotted against the percentage infected in 1993. There was no correlation between years in infection levels found in control plots ($r_s = -0.23$; $p = 0.57$), experimental plots ($r_s = -0.095$; $p = 0.80$) or all plots ($r_s = -0.075$; $p = 0.78$).

Post-Release Gypsy Moth Density in Willoughby Marsh

The population density of gypsy moth in Willoughby Marsh decreased between 1992 and 1993 (Figure 18). Of 45 plots censused in 1992, an average of 195.56 ± 426.91 egg masses/ha was found. Estimates ranged from 0-2200 egg masses/ha. Of 73 plots censused in 1993, an average of 8.22 ± 59.52 egg masses/ha was found. Estimates ranged from 0-500 and egg masses were found in only two plots. There were significant differences in density between 1991 and

Figure 22. Scatter diagram showing the relationship between the percentage of individuals infected with E. maimaiga and density in 15 plots in Willoughby Marsh in 1992.

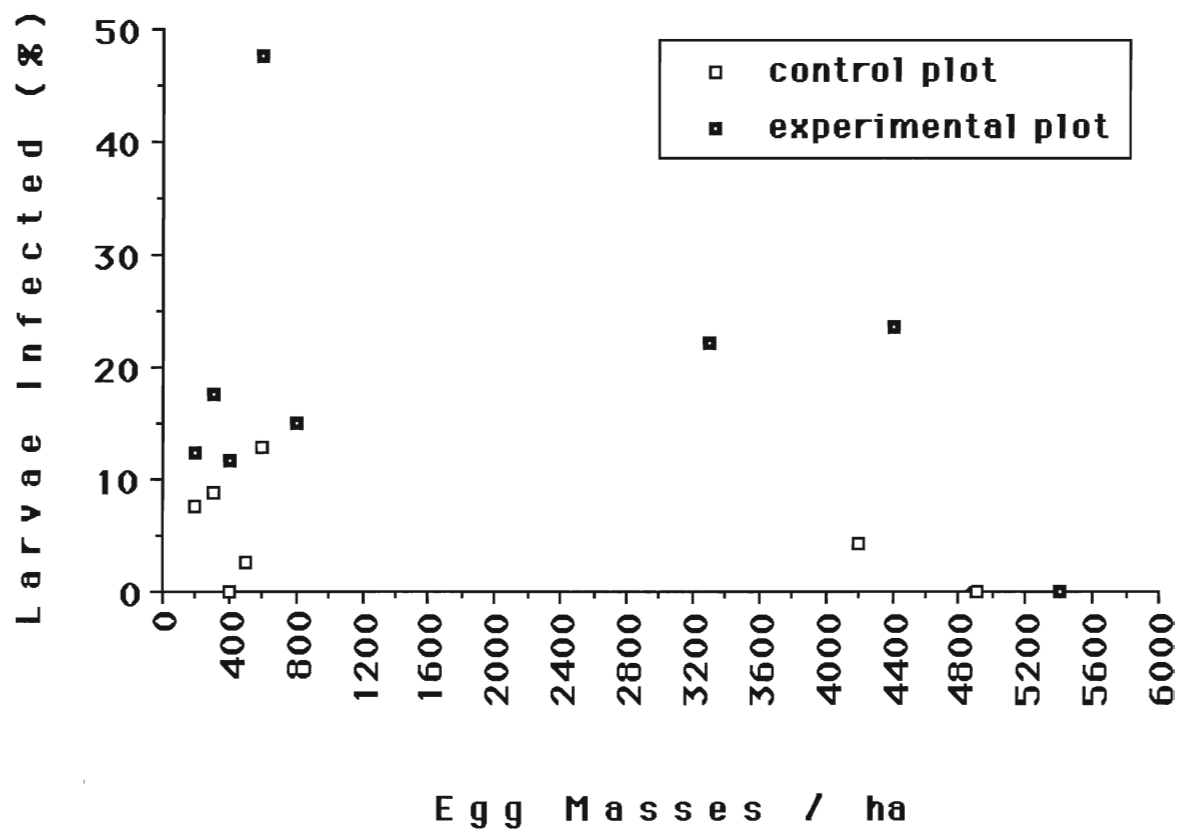


Figure 23. Scatter diagram showing the relationship between the percentage of individuals infected with E. maimaiga and density in 15 plots in Willoughby Marsh in 1993.

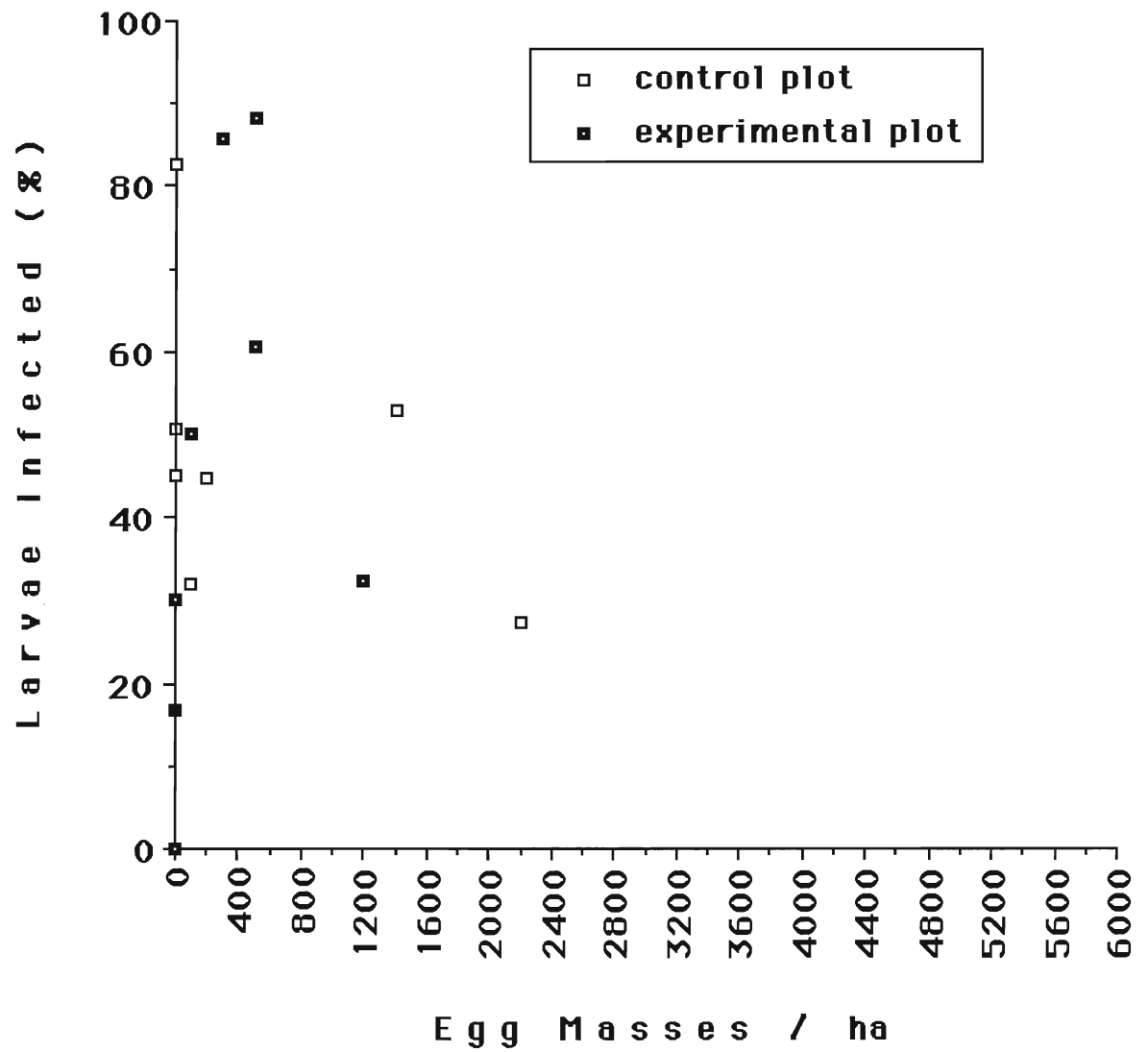
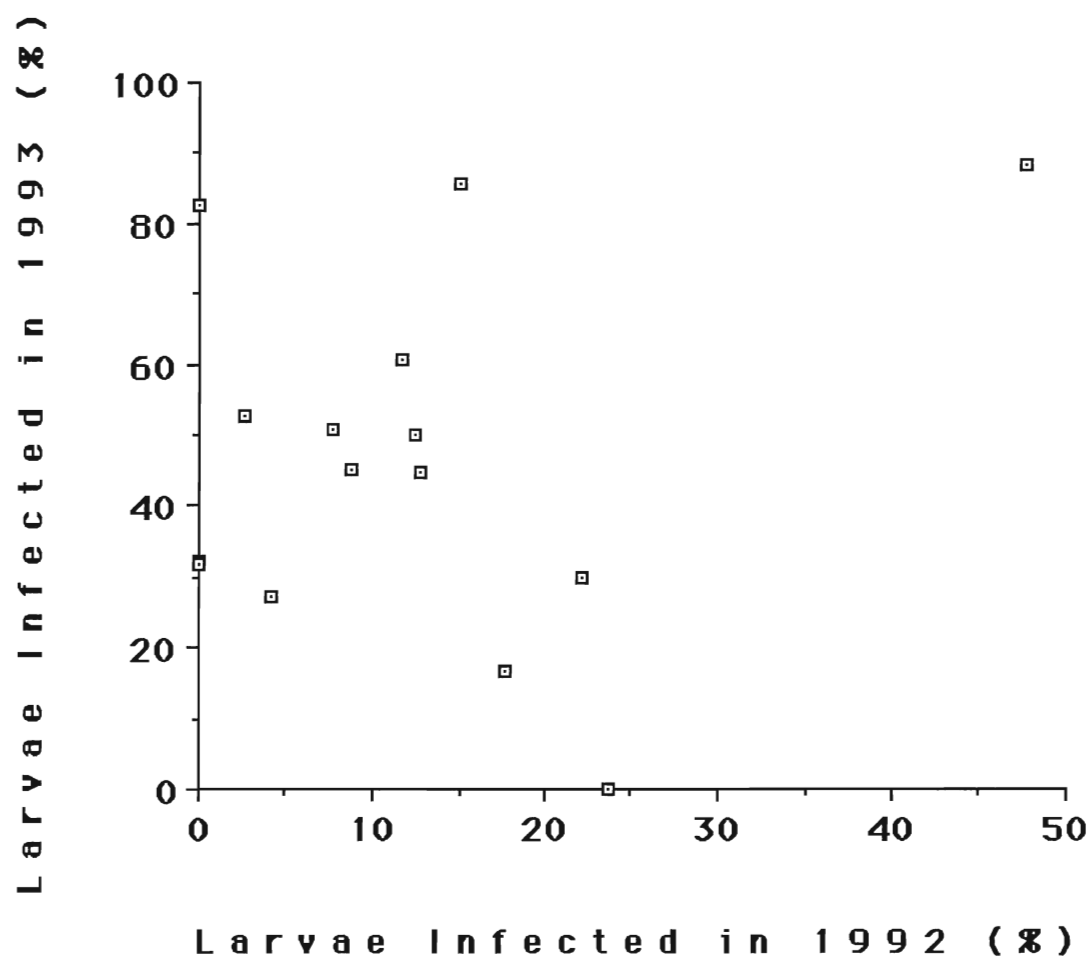


Figure 24. Scatter diagram showing the relationship between the percentage of individuals infected with E. maimaiga from the same plots in Willoughby Marsh in 1992 and 1993.



1993 ($H=43.96$; $df=2$; $p=0.001$). Density in 1991 and in 1992 was not significantly different ($Q=2.3$; $k=3$; $p=0.075$) but density in both of the early years were significantly different from that in 1993 ($Q>3.6$; $k=3$; $p<0.001$).

Mean density estimates obtained from the 15 study plots in Willoughby Marsh between 1991 and 1993 were 1813.33 ± 1984.18 , 433.33 ± 658.64 and 40 ± 129.84 egg masses/ha respectively. Frequency histograms of gypsy moth density in the 15 plots over three years are illustrated in Figure 25. There were significant differences in density between 1991 and 1993 ($H=23.63$; $k=3$; $p=0.0001$). There was a significant drop in density between 1991 and 1992 ($q=3.64$; $k=3$; $0.025 < p < 0.05$) and between 1991 and 1993 ($q=6.60$; $k=3$; $p<0.001$). Density in 1992 and in 1993 was not significantly different ($q=2.96$; $k=3$; $p>0.05$).

Density in fifteen plots chosen in 1992 on the basis of having low, medium or high density did not differ significantly in 1993 ($H=4.17$; $df=2$; $p=0.12$).

Figure 26 shows frequency distribution histograms of population density in the seven control and eight experimental plots in 1993. Density in control plots was not significantly different from that in experimental plots ($U'=36$; $p=0.12$).

An average of 83.33 ± 203.75 egg masses/ha was estimated for Chippawa Creek in 1992. Estimates ranged from 0-500 egg masses/ha. Population density was significantly higher in 1991 than in 1992 ($q=3.4$; $k=3$; $p=0.002$) or 1993 ($q>3.6$; $k=3$; $p<0.001$). No egg masses were found in Chippawa Creek in 1993.

Figure 25. Frequency distribution of gypsy moth egg mass density in 15 plots in Willoughby Marsh Conservation Area in 1991, 1992 and 1993.

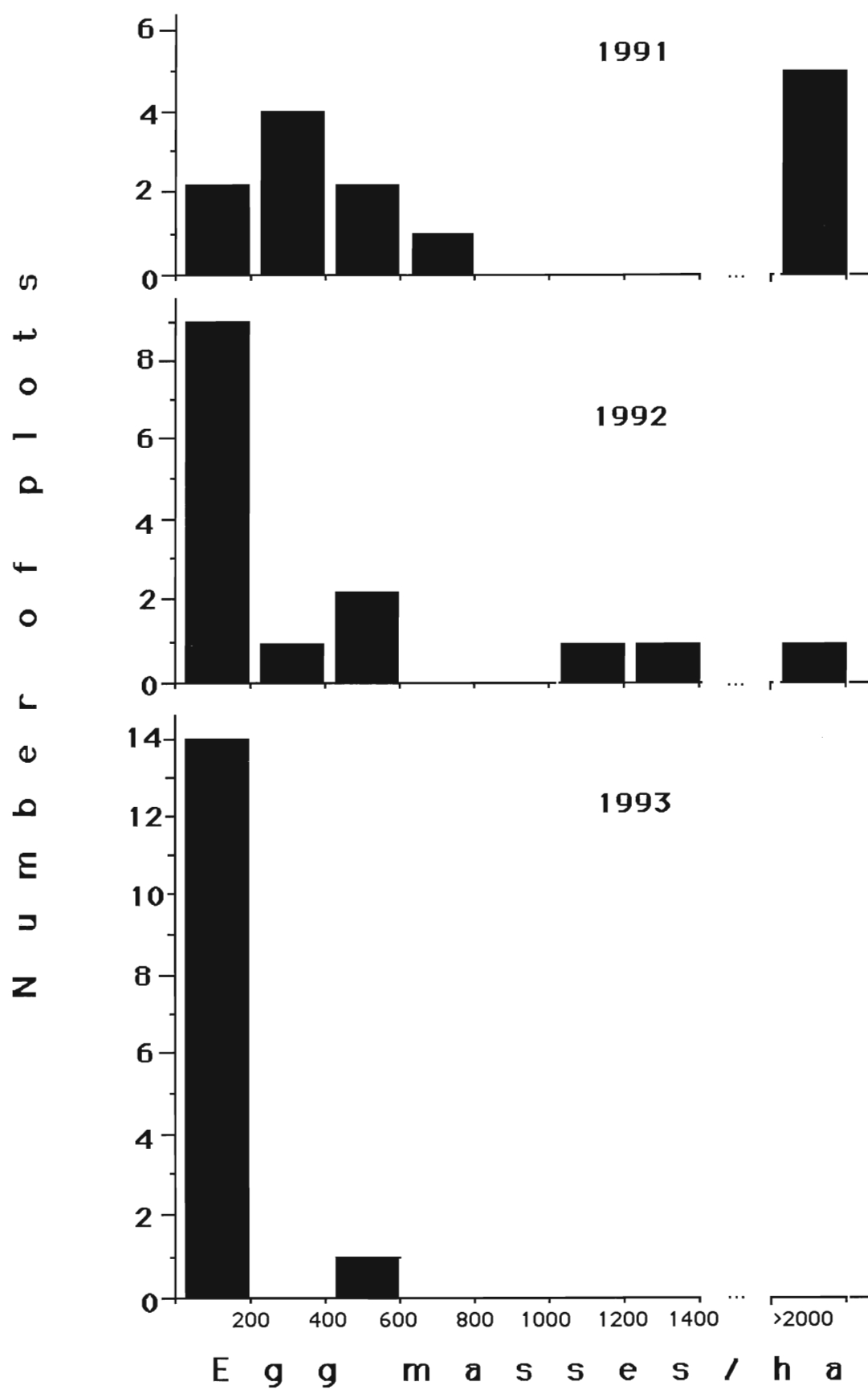
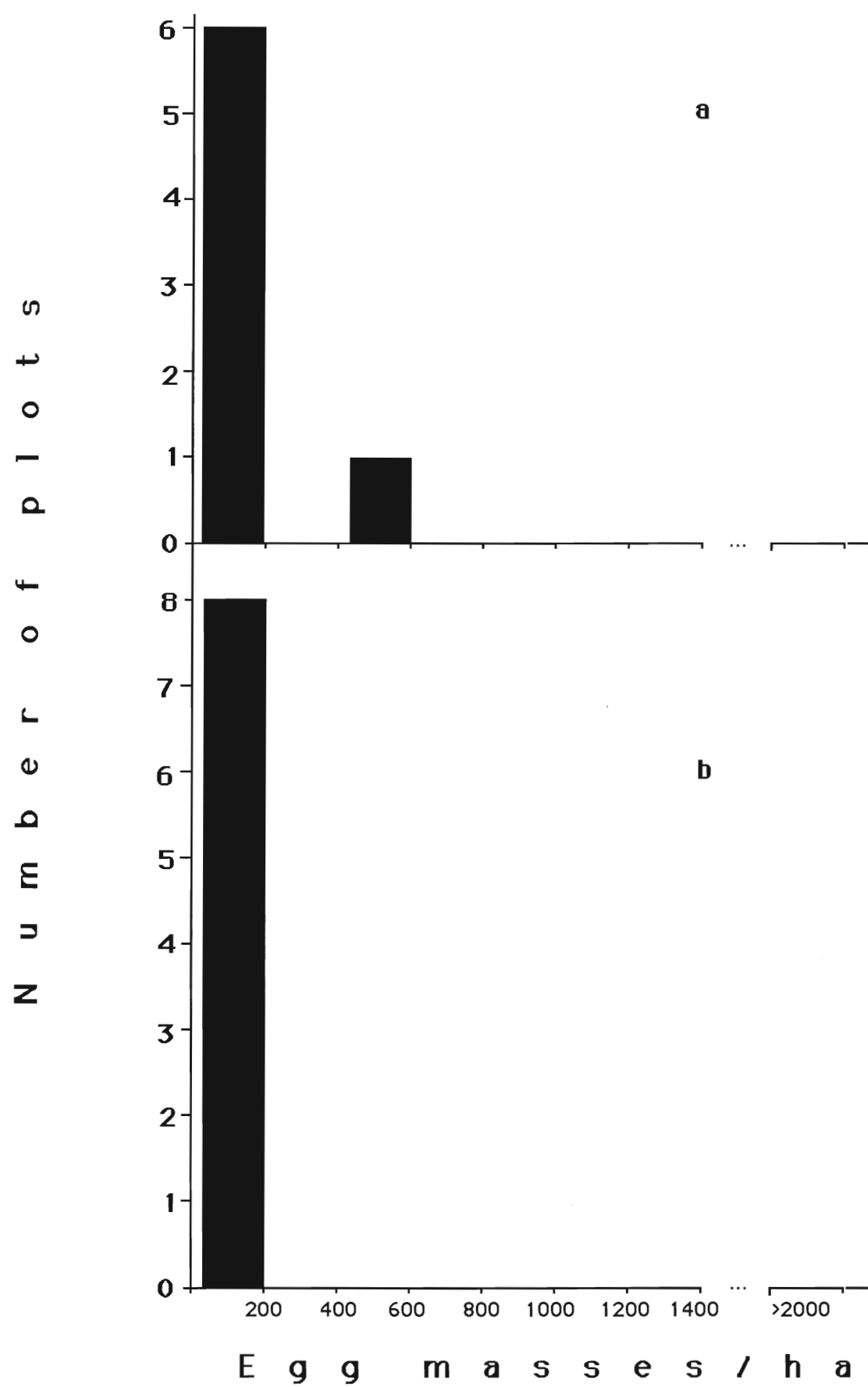


Figure 26. Frequency distribution of gypsy moth egg mass density in (a) control and (b) experimental plots in Willoughby Marsh Conservation Area in 1993.



Occurrence of Nuclear Polyhedrosis Virus

Both in 1992 and 1993, subsets of L. dispar cadavers from each of 15 plots in Willoughby Marsh were also examined for presence of gypsy moth NPV. In 1992, 86.32% of the gypsy moth cadavers examined were positive for NPV (n=101). Larvae positive for E. maimaiga showed a viral incidence of 86.67% from control plots (n=15) and of 92.86% from experimental plots (n=42). Larvae negative for E. maimaiga showed a viral incidence of 85.71% from control plots (n=28) and of 78.13% from experimental plots (n=32). These data are illustrated in Table 6. Presence or absence of NPV was independent of presence or absence of E. maimaiga ($\chi^2=8.52$; v=4; p=0.078).

Table 7 demonstrates how the mean number of PIBs (polyhedral inclusion bodies) per microscopic field per cadaver varied across control and experimental plots of varying density in 1992. Using a non-parametric two-factor ANOVA it was determined that the mean number of PIBs was not affected by whether the larvae originated from control or from experimental plots (F=0.57; df=1; p=0.45) or whether the larvae were infected with E. maimaiga or not (F=0.37; df=1; p=0.54). No association was detected between plot type, fungal status and the mean number of PIBs (F=1.57; df=1; p=0.21).

There was a lower incidence of NPV in 1993 when only 9.62% of gypsy moth cadavers were positive (n=104). Larvae positive for E. maimaiga showed a viral incidence of 6.25% from control plots (n=32) and of 21.74% from experimental plots (n=23). Larvae negative for E. maimaiga showed a viral incidence of 0.04% from control plots (n=25) and of 8.33% from experimental plots (n=24). These data are illustrated in Table 8. Presence or absence of NPV was independent of presence or absence of E. maimaiga ($\chi^2=5.47$, v=4; p=0.25).

Table 6. Incidence of NPV-infected larvae and pupae, positive (+) or negative (-) for *E. maimaiga* from control and experimental plots of varying density in Willoughby Marsh Conservation Area in 1992.

Control			Experimental		
LOW DENSITY			LOW DENSITY		
Plot	+(%)	- (%)	Plot	+(%)	- (%)
C1	75.00(4)	100.00(4)	E4	100.00(5)	100.00(4)
C2	75.00(4)	75.00(4)	E5	100.00(5)	100.00(4)
C3	n/a	75.00(4)	E11	66.67(3)	75.00(4)
MEDIUM DENSITY			MEDIUM DENSITY		
Plot	+(%)	- (%)	Plot	+(%)	- (%)
C6	100.00(4)	100.00(4)	E8	90.00(10)	75.00(4)
C13	100.00(1)	75.00(4)	E14	100.00(6)	50.00(4)
HIGH DENSITY			HIGH DENSITY		
Plot	+(%)	- (%)	Plot	+(%)	- (%)
C7	100.00(2)	75.00(4)	E9	100.00(4)	100.00(4)
C10	n/a	100.00(4)	E12	n/a	100.00(4)
			E15	88.89(9)	25.00(4)

(n) = number of cadavers examined.

Table 7. Mean number of viral polyhedral inclusion bodies per field in gypsy moth larvae and pupae, positive (+) or negative (-) for E. maimaiga from control and experimental plots of varying density in Willoughby Marsh Conservation Area in 1992.

Control			Experimental		
LOW DENSITY			LOW DENSITY		
Plot	+	-	Plot	+	-
C1	0.63(4) ±0.56	4.08(4) ±3.45	E4	95.40(5) ±177.75	9.43(4) ±14.35
C2	29.68(4) ±44.73	47.50(4) ±89.89	E5	149.38(5) ±185.90	96.30(4) ±182.57
C3	n/a	78.30(4) ±147.99	E11	0.27(3) ±0.23	23.43(4) ±46.25
MEDIUM DENSITY			MEDIUM DENSITY		
Plot	+	-	Plot	+	-
C6	51.75(4) ±59.00	65.38(4) ±112.93	E8	73.74(10) ±158.86	19.45(4) ±38.77
C13	3.63(1) ±0.00	43.40(4) ±50.25	E14	47.32(6) ±11.21	0.28(4) ±0.49
HIGH DENSITY			HIGH DENSITY		
Plot	+	-	Plot	+	-
C7	13.25(2) ±17.47	0.05(4) ±0.10	E9	72.55(4) ±119.05	30.90(4) ±18.74
C10	n/a	11.93(4) ±13.95	E12	n/a	11.15(4) ±14.44
			E15	2.36(9) ±2.07	21.93(4) ±43.85

(n) = number of cadavers examined.

Table 8. Incidence of NPV-infected larvae and pupae, positive (+) or negative (-) for *E. maimaiga* from control and experimental plots of varying density in Willoughby Marsh Conservation Area in 1993.

Control			Experimental		
LOW DENSITY			LOW DENSITY		
Plot	+(%)	- (%)	Plot	+(%)	- (%)
C1	0.00(6)	0.00(5)	E4	100.00(2)	0.00(3)
C2	25.00(4)	25.00(4)	E5	12.50(8)	0.00(3)
C3	0.00(6)	0.00(2)	E11	0.00(1)	33.33(3)
MEDIUM DENSITY			MEDIUM DENSITY		
Plot	+(%)	- (%)	Plot	+(%)	- (%)
C6	16.67(6)	0.00(4)	E8	0.00(7)	33.33(3)
C13	0.00(7)	0.00(4)	E14	0.00(1)	0.00(1)
HIGH DENSITY			HIGH DENSITY		
Plot	+(%)	- (%)	Plot	+(%)	- (%)
C7	0.00(1)	0.00(3)	E9	50.00(2)	0.00(3)
C10	50.00(2)	0.00(3)	E12	50.00(2)	0.00(5)
			E15	n/a	0.00(3)

(n) = number of cadavers examined.

Table 9 demonstrates how the mean number of PIBs per microscopic field per cadaver varied across control and experimental plots of varying density in 1993. The mean number of PIBs was not affected by whether the larvae originated from control or experimental plots ($F=1.83$; $df=1$; $p=0.18$) or whether the larvae were infected with E. maimaiga or not ($F=0.24$; $df=1$; $p=0.63$). Interactions between plot type and fungal status did not affect the prevalence of PIBs ($F=0.25$; $df=1$; $p=0.62$).

Adult Sex Ratio

Several gypsy moths, collected in Willoughby Marsh and Chippawa Creek, eclosed to adulthood in the laboratory. Data concerning numbers and sex ratio are given in Appendices 2 and 3.

Parasitoids

Information concerning hymenopteran and dipteran parasitoids which emerged from gypsy moth larvae collected in Willoughby Marsh and Chippawa Creek are given in Appendix 4.

Table 9. Mean number of viral polyhedral inclusion bodies per field in gypsy moth larvae and pupae, positive (+) or negative (-) for E. maimaiga from control and experimental plots of varying density in Willoughby Marsh Conservation Area in 1993.

Control			Experimental		
LOW DENSITY			LOW DENSITY		
Plot	+	-	Plot	+	-
C1	0.00(6)	0.00(5)	E4	141.15(2) ±188.59	0.00(3)
C2	0.025(4) ±0.05	51.43(4) ±102.85	E5	21.11(8) ±59.72	0.00(3)
C3	0.00(6)	0.00(2)	E11	0.00(1)	25.10(3) ±43.47
MEDIUM DENSITY			MEDIUM DENSITY		
Plot	+	-	Plot	+	-
C6	46.35(6) ±113.04	0.00(4)	E8	0.00(7)	513.87(3) ±890.04
C13	0.00(7)	0.00(4)	E14	0.00(1)	0.00(1)
HIGH DENSITY			HIGH DENSITY		
Plot	+	-	Plot	+	-
C7	0.00(1)	0.00(3)	E9	187.20(2) ±264.74	0.00(3)
C10	0.45(2) ±0.64	0.00(3)	E12	0.25(2) ±0.35	0.00(5)
			E15	n/a	0.00(3)

(n) = number of cadavers examined.

Discussion

Gypsy Moth Population Density Estimates

The gypsy moth is considered to be a major defoliator. It has caused extensive damage in the Niagara Region in recent years. During the late 1980s and early 1990s, when control programs were financed by the government, gypsy moth populations declined in those woodlots that were sprayed with Bt. Money to fund gypsy moth spraying programs was withdrawn by the Ontario provincial government in 1993 (Robertson, pers. comm.). Other more self-sustaining methods to manage gypsy moth populations should be investigated. This study examined fluctuations in gypsy moth population density in the Niagara Region and determined whether it was possible to introduce artificially a specific entomopathogenic fungus into local populations. The outcome of these release experiments is discussed followed by an analysis of the potential of using this fungus as a major biological control agent of gypsy moth.

Egg mass surveys performed in this study in 1991 indicated that high variation existed among gypsy moth populations in the Niagara Region. One explanation for this high variation may be that, since within-site variability was high, between-site variability might also be expected to be high. A number of other factors, *i.e.* size of site, topography, food type, occurrence and efficacy of spraying, or a combination thereof, may have contributed to this high variability in density among sites. Some of the variation in gypsy moth density may have been associated with study site size. The three smallest sites had the lowest densities, ranging from 0 to 200 egg masses/ha, and the three largest sites had the largest densities, ranging from 0 to more than 3000 egg masses/ha. Differences in topography may explain

differences in abundance of gypsy moth in certain areas (Weseloh and Andreadis, 1992a). Food type was not likely an important factor in determining density in this study since all six sites contained tree species preferred by gypsy moth. Occurrence of spraying may not have been a major factor because all sites were sprayed to some extent with Bt in 1990 and 1991 (Boles, Hachey and Robertson, pers. comms.). However, the efficiency of the spraying programs may have varied among sites because of differing site characteristics. Occurrence of either high or low density among the six study sites indicates that significant defoliation probably occurred in certain areas of the Niagara Region but not in others in 1991.

For whatever reasons, a certain combination of characteristics common to Shorthills, Chippawa Creek and Willoughby Marsh, led to the survival of some gypsy moths in protected habitats. This combination of characteristics may be absent from Queenston, Niagara-on-the-Lake and Louth, the three sites that supported the lowest populations of gypsy moth. It is likely that when gypsy moth populations again begin to increase from innocuous to outbreak phases, small populations of gypsy moths sheltering in such favourable habitats as Willoughby Marsh and Chippawa Creek will disperse and re-infest other forested locales.

Gypsy moth population densities decreased significantly in Willoughby Marsh and Chippawa Creek during the years that this study was conducted. A decrease in numbers in Willoughby Marsh might have been expected because of the release of an entomopathogenic fungus in 1991 (see later sections) but such a decline was not predicted for Chippawa Creek. The decline in Chippawa Creek and perhaps even in Willoughby Marsh may have been a reflection of a general overall

decrease in gypsy moth populations within the Niagara Region. Provincial and federal authorities had predicted that gypsy moth population declines were likely in many of the heavily infested areas in Ontario, including Niagara. These predictions were based on diminishing size of egg masses deposited in 1991. Small egg masses are indicative of an imminent population decline (Nealis and Erb, 1993). R-strategists, such as the gypsy moth, are frequently suppressed by density independent factors. Density independent factors such as unfavourable weather and/or shortage of food might have led to decreased gypsy moth populations in Niagara and across the province (Evans, 1984).

A disproportionate number of egg masses can be found on over-mature oak trees which are anomalies within the forest (Robertson, pers. comm.). There was such an oak in one plot in Willoughby Marsh and this tree was one of two places in which egg masses were found in 1993. The other place where egg masses were found was on a stunted black willow tree situated along a marshy corridor. These places are significant for future management of the Marsh because this study has identified them as reservoirs of gypsy moths in years when densities are otherwise regionally low. This study showed that specific sites can support high populations of gypsy moth even during times when regional populations are declining.

Egg mass counts may not provide accurate estimates of gypsy moth density when moth populations are in the innocuous stage. During the latter two years of this study, when population density estimates were low, larvae were observed in plots where no egg masses had been detected during the previous fall/winter census. There are two explanations to account for the presence of larvae in

plots previously categorized as having none. First, a few egg masses may have been deposited in cryptic locations and thus were undetected. Second, first or late instar larvae may have dispersed or migrated into some plots. Another method of estimating density, such as larval counts on terminal branches of favoured food plants (Weseloh and Andreadis, 1992a) might provide a better estimate of population density. Although there may be some constraints associated with using egg mass counts as an estimate of population density, these counts are used exclusively in Ontario to determine which woodlots should be treated with insecticide. Egg mass counts are used in many studies, including this one, because of the many advantages associated with this method of estimating gypsy moth density (see Literature Review p. 30-31).

Pathogenicity of E. maimaiga in the Laboratory

Pathogenicity of a culture of E. maimaiga was tested in the laboratory in order to establish its suitability for use in a field release trial. Mean time to death of larvae injected in the laboratory with protoplasts of E. maimaiga isolate ARSEF 2779 was 8.8 ± 2.8 (SD) days. Time to death was comparable though slightly longer than that observed by other researchers injecting a different isolate under similar environmental conditions. In a laboratory study, a Japanese isolate of E. maimaiga, ARSEF 1400, caused death in 7.2 ± 0.2 (SE) days (Hajek et al., 1990b). In another study, Soper et al. (1988) noted that larvae which had been infected with ARSEF 1400 in the laboratory died in a mean time of 5.2 ± 0.1 (SE) days. No information is given about the history of subculturing the test isolate in the Soper et al. study. Hajek et al. (1990b) hypothesized that repeatedly subculturing E. maimaiga in the laboratory results in a decline in virulence. The test isolate

used in the Hajek et al. (1990b) study had been subcultured 15 times which may have led to a decline in virulence and a subsequently longer time to death. Protoplasts used for injection in this study had been sub-cultured seven or eight times which likely led to a corresponding decrease in virulence and an increased time to death among larvae injected in the laboratory.

In this study, E. maimaiga, usually present in the form of resting spores, was found in 64.1% of late instar larvae injected and maintained in the laboratory. Resting spores, the life cycle stage most often observed microscopically in cadavers examined in this study, were identified by Hajek et al. (1990b) as being the predominant life cycle stage which occurs after infection with multiply-subcultured fungal isolates. Hajek et al. found that after 25 transfers of culture medium about 80% of infections produced only resting spores.

The number of protoplasts injected into each larva and percent relative humidity (RH) during the period of disease incubation may affect the amount of ensuing mortality. Larvae in this study were injected with 400-600 protoplasts in approximately 8.4 μ l of culture medium, a dose comparable to that used in other studies. Low RH in the laboratory, about 50%, may have been partially responsible for a smaller proportion of infected larvae dying, only 64%. Shimazu, (1987) observed that larvae injected in the laboratory with 500-1000 protoplasts in 5-10 μ l of culture medium and maintained under dry and humid conditions exhibited 35 to 70% and 0 to 91% infection respectively. Shimazu and Soper (1986) injected 100 protoplasts per larvae in 10 μ l of culture medium and observed 80% mortality at both 93% and 100% RH. Soper et al. (1988) observed close to 100%

mortality in third instar larvae infected with 100 protoplasts. Percentage RH was not reported in this latter study.

In this study, a number of aliquots from the same source were produced and used to inject larvae which were subsequently housed in the laboratory or caged in the field (see next section). Variability between mortality rates in the laboratory and in the four field cages may have been partially caused by the variability which exists in the virulence of samples originally taken from one protoplast culture. Hajek et al. (1990b) observed that cultures derived from the same source and transferred 15 times caused death in varying times. These researchers hypothesized that different fungal aliquots may have a different character and a different absolute response to repeated sub-culturing. Characteristics of different aliquots used in this study may have contributed to the variance in mean times to death in the laboratory and in the field cages.

Survival of Non-Infected and Infected Larvae in Cages

Survival rates of groups of non-infected (medium-injected) and infected (protoplast-injected) larvae housed separately under natural environmental conditions in Willoughby Marsh were recorded. It was anticipated that inferences could be made from data collected on the survival rate of non-infected larvae about the impact of natural conditions on laboratory-raised larvae which had been subjected to an injection regime. Unfortunately over half of the non-infected larvae died within two days of being placed into a cage. Ants observed within this cage may have been responsible for much of this premature mortality. Ants do attack gypsy moth larvae in forest litter (Weseloh, 1989a) and holes in this cage could have readily allowed access to ants. Predatory actions of ants probably shortened the survival rate of

this group of larvae and thus obscured the effect of environmental conditions.

Ants and other predators may also have been responsible for some early larval deaths noted in experimental Cage 2. Mean times to death in the other two field cages, in which had been placed infected larvae, were lower than that in the laboratory indicating that field conditions were likely more rigorous than those that existed in the laboratory. Percent mortality in each cage was higher than that observed in the laboratory indicating that the fungus may not have been the sole mortality factor active in the field.

Death of the majority of infected caged larvae was an indication that death of the majority of their laboratory cohorts, released into field plots, had likely also occurred. Subsequent transmission of E. maimaiga via conidial sporulation from caged/released larvae to field larvae hopefully occurred and field collections commenced. Using time until death of caged larvae to predict time until death of released larvae is a novel method used in this study to determine accurately timing of field collections.

Experimental Release of the Fungus

Once its pathogenicity had been established in the laboratory, the procured strain of E. maimaiga was introduced within bodies of live gypsy moth larvae into eight experimental field plots in Willoughby Marsh. Non-infected larvae were simultaneously released into seven control field plots. A significant difference in the amount of E. maimaiga infection found between experimental and control plots in Willoughby Marsh in 1992 indicated that the release of fungal-infected caterpillars was a successful way of introducing the pathogen into wild gypsy moth populations. Percentage infection that

occurred when infected larvae were released into experimental plots in this study was 10.8 ± 13.8 (SD)%. A similar field study conducted in New York also found that percent infection was significantly higher in experimental plots when compared to that in control plots (Hajek and Roberts, 1991). Hajek and Roberts (1991) reported 20.2% infection in two experimental plots but did not report individual variation between plots. They speculated that the relatively low percent infection that they obtained might have been partially due to the number of infected larvae that were released. Five hundred larvae released into two plots in the Hajek and Roberts (1991) study were nevertheless more than were released in this study ($n=129$ larvae per plot). In the New York study there was a greater period of time for transmission of the fungus since infected larvae were released in early June, about three weeks earlier than in this study. This may have resulted in a slightly greater proportion becoming infected in the Hajek and Roberts (1991) study than became infected in this study.

Infected larvae were released into only two experimental plots in the Hajek and Roberts (1991) study. There were six other experimental plots where other methods of fungal introduction were performed, e.g. resting spores in soil. A great range was found among mean percent fungal infections obtained using different release techniques: from 20.2% for release of infected larvae to 97.9% for release of resting spores in soil at the base of the tree with moisture added. Release of infected larvae was the least successful method.

Mean percent fungal infection in control plots in 1992 was 5.1 ± 4.8 % in this study. Occurrence of fungus in five out of seven control plots in 1992 was unexpected. There are three possible explanations for the occurrence of E. maimaiga in control plots. Field

workers may have inadvertently contaminated control plots by carrying conidia on boots or equipment, airborne transfer of conidia from experimental to control plots may have occurred and/or the fungus may have occurred at low levels in the Marsh prior to this study. The fungus may have spread northward from New York state into southwestern Ontario in the same manner as it probably spread from New York state into eastern Ontario (Welton, 1991). Hajek and Roberts (1991) also found the fungus at low levels in three of eight control plots. They found overall percentage of fungal infection for control plots was $2.1 \pm 1.2(\text{SE})$. These researchers did not offer any speculations as to why the fungus was found in control plots.

All 15 experimental and control plots were re-examined in 1993 in order to assess the ability of the fungus to overwinter in Willoughby Marsh. Findings revealed that occurrence of E. maimaiga was relatively uniform in all 15 study plots one year after the initial release. Approximately half of the larvae collected were infected whether they were from experimental or control plots. In another field study, Weseloh and Andreadis (1992b) found that a little over 30% of late instar larvae collected from the forest were infected with the fungus. However, it has been reported that percent mortality of late instar larvae may be as high as 100% (Weseloh and Andreadis, 1992b; Reardon and Hajek, 1993). Lower percent mortality found in this study may have been due to characteristics of only moderate pathogenicity of the released isolate. Or, as has been noted, the diminishing size of the gypsy moth population in Willoughby Marsh in 1993 may have led to a reduced number of encounters and the rate of pathogen transmission may have been limited. Weseloh and Andreadis (1992a) concluded that the greater impact of the fungus at higher host

densities is probably due to the greater frequency of encounters of pathogen with host.

E. maimaiga-infected caterpillars were not released into Chippawa Creek. In 1993, this fungus was found there. It was not detected in 1992 but it might have gone unnoticed because it is only visible macroscopically for a short time when conidia are sporulating. In 1992, gypsy moth larvae from Chippawa Creek were only examined macroscopically. Spread of E. maimaiga over distances greater than one-km between consecutive years has been reported (Reardon and Hajek, 1993). Since Willoughby Marsh is 32km from Chippawa Creek the fungus may have existed in places between the two study sites before 1993. The fungus may have been spreading naturally in the area prior to the release operation conducted in this study.

Willoughby Marsh and Chippawa Creek are excellent sites within which the fungus may reside in future years. In the spring, presence of free-running water in transient pools formed by melting snow and spring run-off create optimal conditions for germination of resting spores (Hajek et al., 1990c). As has been noted, these two sites also appear to be reservoirs of gypsy moths during years of harsh environmental conditions. First instar larvae, dispersing from these two sites, could also act as a vehicle for the wider dispersion of E. maimaiga.

Discovery of E. maimaiga in gypsy moth in Willoughby Marsh in 1992 represents the first reported occurrence of this fungus in southwestern Ontario. If the introduced isolate of the fungus survives and propagates in Niagara, as anticipated, it will likely disperse naturally to other parts of the province. Hopefully E. maimaiga will become an integral part of the ecological system that governs population

dynamics of gypsy moth in Ontario. Occurrence of E. maimaiga is an exciting development in effective biological control of gypsy moth in the Niagara Region.

Relationship between Fungal Infection and Host Density

In this study, no relationship was found between occurrence of E. maimaiga and gypsy moth density in Willoughby Marsh. As well, there was no relationship between mean fungal infection among different generations of larvae collected from the same plot in different years, i.e. in 1992 and 1993. Other studies that have also examined the relationship between gypsy moth density and occurrence of fungus have produced varying results. Some research indicates that a relationship may exist. A project undertaken by Andreadis and Weseloh (1990) in Connecticut during the year of the first North American fungal epizootic was an ambitious regional study that did not present rigorous comparison data. Results obtained were ambivalent. Highest prevalence of fungal disease appeared to be where gypsy moth populations were largest. However, the fungus was also found where gypsy moth populations were very low (less than 100 egg masses/ha). Significant declines in gypsy moth egg mass densities were observed in most areas of the state and gypsy moth populations did not expand. The researchers noted that it was difficult to assess the impact that the epizootic had on gypsy moth populations. They pointed out that comparable outbreak populations of gypsy moths in 1980 defoliated more than twice the acreage and, had not the fungal epizootic occurred, defoliation in 1989 may have been much greater. Andreadis and Weseloh (1990) declared that the fungal epizootic prevented expansion of the range of the gypsy moth. However, populations of the pest did not collapse everywhere in 1989 and

increased egg mass densities were recorded along the leading edge of the infestation (Andreadis and Weseloh, 1990). In a later study Weseloh and Andreadis (1992a) observed larval mortality was higher in experimental plots having higher densities of gypsy moth suggesting a density-dependent relationship. However, they also noted a dependence on meteorological conditions and suggested that E. maimaiga might be an effective natural enemy of the gypsy moth only during years when high precipitation occurs and host larvae are abundant. Other research, such as that performed by Hajek et al. (1990a), indicates that a relationship between gypsy moth density and levels of fungal occurrence does not exist. Hajek and Roberts (1991) observed activity of E. maimaiga in low as well as high gypsy moth populations in the field. For all release plots where introductions of E. maimaiga had taken place, no association was found between fungal infection level and gypsy moth density at the start of the season during the year of an artificial introduction. However, they did find a significant negative association between levels of fungal infection and densities of egg masses laid at the end of the season. Mortality among early instars dampened the increase in later instar larvae. They also found a similar negative association between fungal infection and annual change in egg mass density. Percent fungal infection was high when annual egg mass density change was low. However, in most of their plots, gypsy moth populations still increased from the year before. The amount of fungal inoculum Hajek and Roberts (1991) placed into the environment was very small in relation to the high number of gypsy moths in the study area. These researchers surmised that high density gypsy moth populations may have overwhelmed the small fungal introduction site and rendered their introduction of the

fungus relatively ineffective (Hajek and Roberts, 1991). Elkinton et al. (1991) also noted no relation between the proportion of dead larvae killed by the fungus and host density. Since E. maimaiga can cause high mortality even at low host population density, it may have the potential to help maintain gypsy moth populations in the low density endemic phase. Lastly Reardon and Hajek (1993) noted that this fungus is prevalent in low-to-high density gypsy moth populations.

Studies that have attempted to define the relationship between gypsy moth density and percent fungal infection have, unfortunately, all used a low number of study plots. Hajek and Roberts (1991) used one or two plots per treatment. Weseloh and Andreadis (1992b) used one to four plots per location. Conclusions derived from data collected from a larger number of experimental plots (eight) in this study might be more reliable.

No significant relationship was found between gypsy moth density and levels of fungal infection in this study. However to state unequivocally that no relationship exists perhaps oversimplifies a very complex association between pathogen abundance and host density. If a relationship does exist it is probably influenced by many variables e.g. food availability, diet quality, soil moisture, rainfall and activity of other pathogens which vary year to year. Since no relationship was found host density may not be an important factor when choosing introduction sites for the fungus.

Nuclear Polyhedrosis Virus (NPV)

In this study, the relationship between two important gypsy moth pathogens, E. maimaiga and NPV, was examined. Other studies have noted only presence/absence of fungus and virus. This study also

examined the relationship between occurrence of E. maimaiga and occurrence and intensity of viral infection.

NPV is considered a pathogen of high density gypsy moth populations (Campbell and Podgwaite, 1971; Dwyer and Elkinton, 1993). The decrease in viral prevalence in late instar larvae in Willoughby Marsh between 1992 and 1993, from 86% to 9.6%, may therefore reflect the observed decline in host population density discussed previously. Campbell and Podgwaite (1971) also determined levels of virus found in high and low density gypsy moth populations. They reported lower percentages of viral infection, *i.e.* 10.6% and 0.5% of gypsy moth larvae were infected with NPV in high and low density populations respectively, in much higher (>10,000 egg masses/ha) and in comparably low (<63 egg masses/ha) gypsy moth populations. An explanation for discrepancies in levels of virus found in these two studies is unclear.

Andreadis and Weseloh (1990) reported that infection with NPV was rare during the first noted fungal epizootic in North America in 1989. The virus was found in only two of 151 sampled locations, and in both cases no fungal infections were observed in the same sites. Viral prevalence may have remained low during the next years of fungal epizootics in New England. One field study determined that mean weekly virus prevalence was around 2% in 1990 and less than 15% in 1991 (Weseloh and Andreadis, 1992a). Elkinton *et al.* (1991) observed that none of the larvae they sampled for fungus died of NPV. Other researchers have found evidence of both of these pathogens within the same population of gypsy moths. Percentages of larvae infected with E. maimaiga and NPV ranged between 19.4 and 47.6% ($X=38.1\pm9.3\%(SE)$) and 43.8 and 64.7% ($X=54.6\pm6.0\%(SE)$) respectively in

New York in 1991 (Hajek and Roberts, 1992). Both of these pathogens were found in Willoughby Marsh during 1992 and 1993. Percentages of larvae infected with E. maimaiga and NPV ranged between 0 and 47.6% ($X=12.4\pm12.4\%(SD)$) and 78.1 and 92.9% ($X=85.8\pm19.2\%(SD)$) respectively in 1992. Percentages of larvae infected with E. maimaiga and NPV ranged between 0 and 85.7% ($X=46.5\pm25.3\%(SD)$) and 0.04% and 21.74% ($X=13.6\pm23.9\%(SD)$) respectively in 1993.

Mixed infections involving two or more pathogens of gypsy moth within the same individual are not unknown. For example Aoki (1974) reported mixed infections of E. maimaiga and Paecilomyces canadensis (Vuill.) Brown & Smith from gypsy moths in Japan. In this study, mixed infections of the fungus and NPV were found in 44.4% and 7.7% of cadavers examined in 1992 and 1993 respectively. Hajek and Roberts (1992) found mixed infections of E. maimaiga and NPV in 4.4% of gypsy moth larvae analyzed. Larvae infected with both pathogens die faster than larvae infected with only one (Hajek, pers. comm).

In this study, no relationship was found between occurrence of NPV and occurrence of fungus in gypsy moth cadavers from either control or experimental plots in Willoughby Marsh in 1992 and 1993. In other words it was just as likely for a larva that was infected with E. maimaiga as a larva that was not infected to contain viral particles. It may be concluded that neither the fungus nor the virus appeared to inhibit activity of the other within the same individual nor to predispose the individual to infection by the other pathogen.

In this study, no relationship was found between intensity of viral infection and occurrence of fungus in either control or experimental plots in Willoughby Marsh in 1992 and 1993. The fungus was able to penetrate hosts that were lightly and heavily infected

with virus. This finding, which suggests a lack of competition between two pathogenic organisms for a limited host resource, is not puzzling because this fungus/virus system is not at equilibrium.

There are three seemingly superior characteristics of fungus over virus. E. maimaiga infects its host through contact with the integument. NPV must be ingested. E. maimaiga kills its host in about a week. NPV takes about two (Woods and Elkinton, 1987). Penetration by a single conidium likely leads to death of a larva (Hajek, pers. comm.). NPV may be present at sub-lethal levels without killing the host. It would seem that those larvae infected with both pathogens at the same time would succumb first to fungal infection.

Conclusions

Although the introduction of E. maimaiga in this study was successful, only a moderate proportion of gypsy moth larvae in the 15 study plots became infected during the year of the release. The method of fungal release used in this study was extremely time-consuming and labour-intensive. Every larva had to be handled individually and injected with care. Injecting larvae with protoplasts and then releasing them was the least successful of four introduction methods used by Hajek and Roberts (1991). Omission of discussion of this method in a review by Reardon and Hajek (1993) likely indicates that it will not be considered for future large-scale introduction projects. Other methods of artificial release are more efficient and can cause a higher infection levels. For example, 97.9% fungal infection occurred when spores were placed in soil at the base of a tree and moisture added (Hajek and Roberts, 1991). Utilization of the methodology used in this study is not recommended for practical use

by woodlot managers. Other methods are easier to perform and produce higher mortalities (Hajek and Roberts, 1991).

Different life stages of E. maimaiga may all be used as a means of artificially transferring fungi from one site to another. Some, e.g. conidia, are more difficult to manipulate than others, e.g. resting spores. Entomophthoralean conidia are not a suitable life cycle stage for use in control of gypsy moth because, due to their fragility and short lifespan, they must be stored under finely defined environmental conditions. Resting spores are easier to store for long periods and are resistant to drought and other adverse conditions in the environment. They are considered an excellent life stage for use in control of certain pests (Hajek and Reardon, 1993). Entomophthoralean resting spores have been successfully used against aphids. For instance, mass production of resting spores of the fungus, Erynia thaxteriana, has been accomplished with greater than 98% successful germination (Soper et. al., 1975). The ability of the fungus, E. maimaiga, to produce resting spores was considered by Speare and Colley (1912) as being a highly desirable characteristic of this pathogen. They believed that "should it (gypsy fungus) obtain a foothold in the field ... it might be expected to prove continuously effective from season to season, owing to its' habit of forming resting spores in great abundance, which the experiments have shown are able to survive the New England winter...". Despite these encouraging remarks, resting spores of E. maimaiga, in contrast to those of other entomophthoraleans, are not well suited for artificial manipulation for control of the gypsy moth. Most attempts to induce epizootics using resting spores have been unsuccessful (Reardon and Hajek, 1993). It is difficult to produce large quantities of resting spores because of the practical constraints of growing E.

maimaiga in artificial medium. It is difficult or impossible to induce their germination (Soper *et al.*, 1975). Requirements for germination of resting spores of E. maimaiga in the laboratory are so far unknown. Attempts to increase the prevalence of E. maimaiga in an area by transplanting overwintering resting spores may be unsuccessful because of threshold effects, that is there must be a certain amount of inoculum, a measurement of which is unknown, in an area to trigger an epizootic (Hajek and Roberts, 1991; Weseloh and Andreadis, 1992b). And even if enough resting spores are released into the environment, germination will not occur in the absence of sufficient precipitation. Although it would be impractical, Weseloh and Andreadis (1992b) suggested that sprinkler irrigation might succeed on small sites perhaps in conjunction with augmentation of resting spores

Mycelia may also be used in control of insect pests. In the United States, the fungus, Hirsutella thompsonii, is applied as mycelial fragments where it is used successfully against the citrus rust mite, Phyllocoptruta oleivora (McCoy *et al.*, 1972). Mycelia are more easily produced than resting spores (Reardon and Hajek, 1993). Mass fermentation techniques have been developed for strains of the species complex of E. aulicae other than E. maimaiga. Strains of E. aulicae can be cultured outside of the host in mycelial form, thus facilitating potential mass production (Tyrrell, 1988). McCabe and Soper (1985) have a patent for producing E. maimaiga as a mycoinsecticide. Only limited trials have been conducted so far but preliminary attempts to grow E. maimaiga in liquid media have been unsuccessful (Soper *et al.*, 1988).

Results from various experiments with E. maimaiga have been highly variable. All of the physiological requirements of the fungus

are still unknown. The fungus does display a great deal of dependence on meteorological conditions and it would therefore not be suitable for all areas of North America. The relationship between pathogen and host density is still unclear. E. maimaiga seems to be a highly unpredictable control agent at low and high gypsy moth densities. At present, because of the constraints involved in producing it in large, viable quantities, E. maimaiga might only be a useful tool for improving biological control of the gypsy moth on a small scale (Weseloh and Andreadis, 1992b). It seems, as yet, debatable whether it can be effective in reducing gypsy moth populations on a larger scale. Due to the cyclic nature of gypsy moth population dynamics, gypsy moth populations in Ontario will likely increase in the next few years and spark renewed interest in the practicality of performing artificial introductions of E. maimaiga.

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APPENDICES

Appendix I
GYPSY MOTH EGG MASS SURVEYS

MODIFIED KALADAR PLOT

PLOT # _____ OWNERSHIP _____ DATE _____
 DISTRICT _____ COUNTY/MUNICIPALITY _____
 TWSP. _____ CON. _____ LOT _____
 SPECIES COMP. SURROUNDING: _____ WITHIN: _____
 CREW _____

EGG MASS COUNTS (new egg masses)

STEP 1) Above Ground Count _____

STEP 2) Mini-Plots :

1 _____ 2 _____ 3 _____ 4 _____ 5 _____

6 _____ 7 _____ 8 _____ 9 _____ 10 _____

Total (add counts from plots 1 to 10) = _____ (GROUP)

Adjusted Ground Count : (GROUND) X 10 = _____

STEP 3) # in MKP _____ (i) + (ii) = _____ (TOTAL)

STEP 4) # Egg Masses/Ha..... (TOTAL) X 100 = _____ EM/HA

Note: If you calculated % new egg masses please record your calculations here:

New _____ # Old _____ % New _____

COMMENTS:

Egg Mass Length (mm)

_____	1 _____	11 _____	21 _____
_____	2 _____	12 _____	22 _____
_____	3 _____	13 _____	23 _____
_____	4 _____	14 _____	24 _____
_____	5 _____	15 _____	25 _____
_____	6 _____	16 _____	26 _____
_____	7 _____	17 _____	27 _____
_____	8 _____	18 _____	28 _____
_____	9 _____	19 _____	29 _____
_____	10 _____	20 _____	30 _____

Average Egg Mass Length = _____

Appendix 2. Number of each sex of gypsy moth adults which emerged from collections made in study plots in Willoughby Marsh Conservation Area in 1992 and 1993.

Site	1992			1993		
	N	Males	Females	N	Males	Females
C1	52	2	1	83	1	2
C2	46	6	3	31	1	0
C3	41	6	0	29	3	0
E4	40	1	1	10	0	0
E5	43	0	1	66	0	0
C6	39	0	0	74	0	0
C7	48	4	2	11	1	0
E8	21	0	1	42	0	0
E9	18	2	1	10	0	0
C10	16	0	0	25	2	0
E11	17	1	2	6	0	0
E12	36	0	1	31	0	0
C13	38	0	0	53	15	4
E14	40	0	0	7	0	0
E15	38	5	1	8	0	0

N = Number of gypsy moth larvae and/or pupae collected from each plot.

Appendix 3

Sex Ratio

Several gypsy moths eclosed to adulthood in Willoughby Marsh in 1992 (n=41) and in 1993 (n=29). The sex ratio was 1:0.5 in 1992 and 1:0.3 in 1993. The number of each sex was not significantly different between years ($\chi^2=1.51$, v=1, p=0.23). Of all the larvae collected in 1992 and 1993, 7.69% and 5.86% respectively eclosed to adulthood in the laboratory.

Of 139 larvae collected in Chippawa Creek in 1993, 7.19% eclosed to adulthood in the laboratory. The sex ratio was 1:0.3.

Appendix 4

Parasitoids

Other causes of mortality such as parasitoids and nematodes that may have affected density levels in the study plots in Willoughby Marsh were not examined in detail in this study. Evidence of them was observed. In the laboratory one dipteran larva usually emerged from each host larva but occasionally two would develop. These parasitoids usually died in the puparial or pupal stage. Dipteran adults ($n = 2$) and puparia ($n = 29$), hymenopteran adults ($n = 4$) (*Anisocyrta* sp.) and pupae ($n = 8$) (Braconidae) emerged from 486 larvae collected from Willoughby Marsh in 1993. Dipteran adults ($n = 3$) and puparia ($n = 10$), hymenopteran adults ($n = 2$) and pupae ($n = 2$) were collected from Chippawa Creek gypsy moth larvae ($n = 139$) in 1993. Many cadavers examined from one of the study plots in Willoughby Marsh were infested with nematodes. Parasitoids have been shown to influence gypsy moth population dynamics. A high incidence of parasitoid involvement occurs in relatively stable sparse populations of gypsy moth (Campbell and Podgwaite, 1971) and in high density populations. In a field study that examined various causes of gypsy moth mortality in the field, Gould *et al.* (1990) observed that high density gypsy moth population experienced highest mortality rates. Density-dependent mortality was caused by the generalist tachinid parasitoid, *Compsilura concinnata*, which was found to aggregate in study plots with high density gypsy moth populations.